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THE LIFE CYCLE OF ARCHIGETES IOWENSIS

(CESTODA: CARYOPHYLLIDEA)

by

Robert Leland Calentine

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INTRODUCTION AND HISTORICAL REVIEW

Caryophyllaeid cestodes are unsegmented helminths possessing a single set of reproductive organs and varying in length from 1 to 75 mm. Adult caryophyllaeids parasitize members of certain families of freshwater teleost fishes, but some species differ from all other cestodes in their ability to become ovigerous in invertebrate as well as in vertebrate hosts. Despite the accumulation of considerable literature dealing with these parasites, their taxonomic relationships remain unclear.

According to Hunter (1930), cestodes now known to be caryophyllaeids were first described from fish by Pallas in 1781 under the name *Taenia laticeps* (later re-determined as *Caryophyllaeus laticeps*). *Caryophyllaeus*, perhaps the best-known genus of this cestode group, was established by Gmelin (1790). Most workers assign authorship of the family Caryophyllaeidae to Leuckart (1878), but Janiszewska (1954) credited Claus (1868) as author of this taxon. However, study of early literature dealing with these cestodes indicates that authorship of this family cannot be properly credited to either Leuckart or Claus. Carus (1863) considered caryophyllaeids as a family, referring to them as "Caryophyllidea van Beneden." Olsson (1893) also attributed authorship of the "family Caryophyllidea" to van Beneden. Although neither investigator listed a specific date of authorship, Carus (1863) cited van Beneden's (1850b and 1861) papers while Olsson (1893) referred to van Beneden's (1861) account. Study of the latter's (1861) paper shows that he divided cestodes into two groups, Digénèses and Monogénèses. In the former group he placed the Téniades and the Bothriadés. The

Bothriadés was composed of three families: Tétraphylles, Diphylles, and Pseudophylles. The Monogénèses contained a single genus "Caryophylleus." Van Beneden (1861) also used the term "Caryophyllé" with reference to caryophyllaeid cestodes. He did not, however, refer to them as a distinct family, nor did he use the name "Caryophyllidea" (see van Beneden's 1849, 1850, and 1861 accounts). Carus (1863) apparently latinized van Beneden's "Caryophyllé" to "Caryophyllidea", which he treated as a distinct family. Since Carus (1863) evidently first erected a family of caryophyllaeids, based upon a genus (Caryophyllaeus) of this group, and since van Beneden has not been generally credited with authorship of this family, the proper familial designation appears to be Caryophyllaeidae Carus, 1863. Leuckart (1878) first used the proper spelling of the family name.

Nybelin (1922) considered caryophyllaeids as a subfamily (Caryophyllaeinae) of his new family, Cyathocephalidae. Mola (1929) treated caryophyllaeid cestodes as an order (Caryophyllidea) of the class Cestodaria Monticelli, 1892. Hunter (1930) considered them as a family (Caryophyllaeidae) of the order Pseudophyllidea Carus, 1863. He distinguished four subfamilies: Caryophyllaeinae Carus, 1863; Capingentinae Hunter, 1930; Lytocestinae Hunter, 1927; and Wenyoninae Hunter, 1927. Hunter attributed authorship of the subfamily Caryophyllaeinae to Nybelin (1922), but this name became available with Carus's (1863) publication.

Hyman (1951) also referred to caryophyllaeids as a family of the Pseudophyllidea, but Wardle and McLeod (1952) treated them as an order. Although they were aware of Mola's (1929) ordinal consideration of these helminths within the Cestodaria, Wardle and McLeod, in their taxonomic revision, included the Caryophyllidea as one of their newly established orders of the class Cestoda Carus, 1863. They also elevated the four subfamilies recognized by Hunter (1930) to familial rank.

Yamaguti (1959) retained the order Caryophyllidea, but in certain places (pages 8 and 451), placed it within the Cestodaria, and at another (page 7), included it within the Eucestoda Southwell, 1930. He distinguished a single family, Caryophyllaeidae, consisting of three subfamilies, namely Caryophyllaeinae, Capingentinae, and Lytocestinae. Yamaguti included cestodes of the genus *Wenyonia* Woodland, 1923, within the Caryophyllaeinae; Wardle and McLeod (1952) considered them as a distinct family, while Hunter (1930) gave them subfamily rank. Yamaguti recognized van Beneden (in Olsson, 1893) as author of the ordinal name, Caryophyllidea. Olsson's (1893) and van Beneden's (1849, 1850, and 1861) considerations of caryophyllaeids have been mentioned previously. The ordinal name "Caryophyllidea" apparently originated with Carus (1863), but caryophyllaeids were first given ordinal rank by Mola (1929).

In more recent accounts, namely those of Joyeux and Baer (1961), Kulakowskaja (1961), and Stunkard (1962), caryophyllaeids are considered as a family of the Pseudophyllidea.

A complete historical treatment of caryophyllaeids is not included in this paper. It is evident that the taxonomy of these parasites should be reconsidered, with special attention given to the rules of nomenclature. For details of early accounts of these parasites, the studies of Braun (1894), Hunter (1930), and Wardle and McLeod (1952) should be consulted.

In this paper, caryophyllaeids are considered as a distinct order, Caryophyllidea, of the class Cestoda. I follow Yamaguti's (1959) taxonomic scheme with respect to family and subfamily arrangement.

Although arrangement of caryophyllaeids into subfamilies is currently based upon the position of the testes and vitellaria with respect to the parenchymal muscles, Szidat (1942) expressed belief that this criterion may be inadequate. Recent studies by Anthony (1958), Janiszewska (1954), and by Mackiewicz (1960) tend to confirm Szidat's viewpoint. At the present time, those caryophyllaeids whose testes and vitellaria are within the medullary parenchyma are assigned to the subfamily Caryophyllaeinae (or to the family Caryophyllaeidae if Wardle and McLeod's taxonomic scheme is followed). Eleven genera are currently assigned to this subfamily: Archigetes Leuckart, 1878; Biacetabulum Hunter, 1927; Bialovarium Fischthal, 1953; Caryophyllaeus Gmelin, 1790; Glaridacris Cooper, 1920; Hypocaryophyllaeus Hunter, 1927; Monobothrium Diesing, 1863; Paraglaridacris Janiszewska, 1950; Pliovitellaria Fischthal, 1951; Szidatinus McCrae, 1961 (=Brachyurus Szidat, 1938); and Wenyonia Woodland, 1923. In addition, Paracaryophyllaeus Kulakowskaja, 1961, may belong to this subfamily.

Members of the genus Archigetes have long been recognized as the only cestodes capable of becoming ovigerous within invertebrate (oligochaete) hosts. More recently, other caryophyllaeid genera have been described whose members are capable of reaching maturity in tubificids, and may even produce eggs in such hosts. Most species apparently become ovigerous only if they reach suitable fish hosts.

The concept of *Archigetes* as a distinct genus has been questioned by several investigators, who believe that species assigned to this genus are progenetic larvae of other genera, such as *Biacetabulum*. Unfortunately, the paucity of experimentally controlled life cycle studies has been a hindrance to the complete understanding of this cestode group.

Caryophyllaeids were first reported from oligochaetes by Udekem (1855) from *Nais proboscidea* O. F. Müller, 1774 and from *Tubifex rivulorum* Lamarck, 1816. (Brinkhurst, 1960, lists *T. rivulorum* as a synonym of *Tubifex tubifex* O. F. Müller). Although the taxonomic position of Udekem's specimens has never been ascertained, it is generally believed that those from *T. tubifex* are larvae of *Caryophyllaeus*.

Ratzel (1868) reported various developmental stages (some mature, but not gravid) of a caryophyllaeid from the tubificid, *T. tubifex* (=*T. rivulorum*) in Germany. He named the parasite *Caryophyllaeus appendiculatus*, placing it in the genus previously established by Gmelin in 1790. Leuckart (1869) considered the cestodes described by Ratzel to be larvae of *Caryophyllaeus laticeps* (Pallas, 1781) (=*C. mutabilis* Rud.). Leuckart later (1878) described, but did not illustrate, a caryophyllaeid species from *T. tubifex* in Germany. He believed his parasites (which included gravid specimens) to represent the same species as that reported by Ratzel in 1868. Leuckart now decided that since these worms possessed a bothriate scolex and became gravid in tubificids, they could not be ascribed to the genus *Caryophyllaeus*. For this reason, he placed these cestodes in a new genus, *Archigetes*, within the family Caryophyllaeidae.

Furthermore, he (page 601) renamed this cestode A. sieboldi. Leuckart, still later, (1886) illustrated this species, but internal details are not clearly distinguishable.

Gruber (1881) accepted A. sieboldi as a valid species, but Braun (1894), using Leuckart's illustration, referred to it as A. appendiculatus (Ratz.).

Mrázek (1897) described gravid specimens of a caryophyllaeid species from T. tubifex and from Limnodrilus claparedianus Ratzel, 1868, in Czechoslovakia, referring to them as A. appendiculatus. He considered this species to be identical to C. appendiculatus and also to Leuckart's A. sieboldi.

The authorship of A. appendiculatus and the possible synonomy of this species with A. sieboldi have been much disputed by other workers. Nybelin (1922) considered Ratzel's specimens to be larvae of Caryophyllaeus. Wisniewski (1930), likewise, did not accept Ratzel's cestodes as members of the genus Archigetes. His reasons for so believing were based upon the fact that these cestodes lacked a bothriate scolex, and were not gravid. Wisniewski believed Mrázek's A. appendiculatus to be synonomous with A. sieboldi. Fuhrmann (1931) credited Ratzel with authorship of A. appendiculatus, but used Mrázek's illustration of this species (page 327). Janiszewska (1950), on the other hand, assigned authorship of A. appendiculatus to Mrázek and held this species to be distinct from A. sieboldi. Wardle and McLeod (1952), accepting Wisniewski's taxonomic disposition of Archigetes, recognized Leuckart's A. sieboldi. Joyeux and Baer (1961) used the name A. sieboldi (page 365) to caption Mrázek's illustration of A. appendiculatus. It is evident that the true identity of these species requires additional study.

Other studies concerning cestodes of the subfamily Caryophyllaeinae have appeared in the literature. Mrázek (1901), in Czechoslovakia, found mature larvae in Tubifex sp. and, after careful study, concluded that they represented C. laticeps. Later (1908), he described Archigetes brachyurus from Limnodrilus hoffmeisteri Claparède and stated that A. appendiculatus was also harbored by this oligochaete.

Ward (1911), in a brief account, reported the discovery of Archigetes in America. His specimen, recovered from a fish, lacked a cercomer. Heretofore, all references to Archigetes involved parasites characterized by the possession of a cercomer and inhabiting tubificid oligochaetes. According to Mackiewicz (1961b), there is some doubt that Ward's specimen was, indeed, Archigetes.

Hunter (1930), also in America, reported obtaining oligochaetes infected with Archigetes, but did not describe these cestodes. Personal communication with Hunter indicates that these specimens are no longer extant.

Wisniewski (1928) described A. cryptobothrius from L. hoffmeisteri in Poland, and later (1930) presented a comprehensive study on the genus Archigetes, including details of experiments involving A. sieboldi. He infected oligochaetes with embryonated eggs taken from parasites within the body cavity of L. hoffmeisteri, thus proving that the life cycle is not dependent upon a fish host. Furthermore, he was unable to infect fish of the genus Carassius with specimens of A. sieboldi from tubificids.

Wisniewski emphasized that *Archigetes* is a distinct genus whose members complete their development in oligochaete hosts, that species of *Archigetes* should be considered as neotenic procercoids (with which stage the life cycle is completed), and that the annelid host acquires its infection through the ingestion of embryonated eggs.

The taxonomic problems indicated above have been compounded by knowledge of related genera whose morphological features closely approximate those of *Archigetes*. This is particularly true of the genus *Biacetabulum*, erected by Hunter (1927) when he described *B. infrequens* from *Moxostoma anisurum* (Raf.) in America. This author (1929) also described *B. meridianum* from *Erimyzon sucetta* (Lacépède) and *B. giganteum* from *Ictiobus bubalus* (Raf.) and *Ictiobus* sp., also in America. Johnston and Muirhead (1950) reported another species of this genus, *B. tandanus*, from an Australian siluroid fish, *Tandanus tandanus* Mitchell. McCrae (1961) reported two undescribed species of this genus from a catostomid fish, *Catostomus commersoni* (Lacépède) in Colorado. Mackiewicz (1961a) found still another undescribed species of *Biacetabulum*, also from *C. commersoni*, in New York.

One species of the genus *Glaridacris* resembles *Archigetes* in that specimens may become ovigerous in annelids. Yamaguti (1934) described *G. limnodrili* from an oligochaete (*Limnodrilus* sp.) and from the fishes *Pseudogobio esocinus* Temminck and Schlegel, and *Misgurnis fossilis* (L.) in Japan. He stated that specimens from oligochaetes were "fully matured" and concluded that such cestodes were able to complete their development and produce eggs in either host.

Szidat (1938) described a related cestode, Brachyurus gobii, from the fish Gobio fluviatilis Cuv. in Germany. He placed Yamaguti's G. limnodrili, as well as Mrázek's A. brachyurus in this new genus. Yamaguti later (1959) considered the genus Brachyurus invalid (because of preoccupation) and transferred G. gobii to Glaridacris. He also placed A. brachyurus in the latter genus. McCrae (1961) proposed the name Szidatinus to replace Brachyurus.

The first evidence that Archigetes might be associated with a fish host came with the publication of Szidat's (1937) study of a caryophyllaeid species from the cyprinid fish Tinca tinca L. in Germany. On the basis of morphological similarities, Szidat thought this species to be identical to A. sieboldi, which previous investigators had found in oligochaetes. Since Szidat's specimens were firmly attached to the fish intestine, he concluded that fish constituted the normal definitive host of A. sieboldi, and that specimens from oligochaetes were but progenetic larvae. He further stated that these cestodes probably remain in fish only a short time and that additional study would disclose that all members of the genus do occur as fish parasites. It should be noted that in this study (1937), Szidat makes no mention of A. appendiculatus. Although he was aware that the genus Archigetes had been established in 1878 (much earlier than Biacetabulum, created in 1927), Szidat nonetheless designated this parasite B. sieboldi. His reason for doing so was that cestodes of the Archigetes-Biacetabulum complex had first been described from fish by Hunter in 1927 under the latter name. Moreover, descriptions of Archigetes, according to Szidat, had been based upon progenetic larvae of more than one genus of fish parasites, including Biacetabulum.

Marcus (1942) reported the presence of A. sieboldi in L. hoffmeisteri and L. udekemianus Claparède, 1862, in Brazil. Although he expressed doubt that Archigetes and Biacetabulum were synonomous, Marcus indicated that if such were the case, Archigetes would be the valid name.

Baer (1952) discounted Szidat's conclusions because of the lack of experimental evidence to support them.

Janiszewska (1950) reported on caryophyllaeid cestodes which she held to be identical to Szidat's B. sieboldi. Her specimens were found in the fishes Barbus barbus (L.), T. tinca, and Abramis brama (L.) in Poland. Janiszewska believed these fish parasites to be adults of A. appendiculatus, not A. sieboldi (as Szidat has maintained from his study of caryophyllaeids from T. tinca) and called them B. appendiculatum. Janiszewska later (1954) re-emphasized her 1950 views and further stated that Archigetes, "is not a genus in the true meaning of the word since it represents the progenetic larval stages of various species and genera" (translation).

Yamaguti (1959), in his account of cestodes of fishes, retained the generic designation Archigetes, but restricted it to "mature procercoids in annelids" and listed sieboldi as a species of Biacetabulum.

Kulakowskaja (1961) reported A. sieboldi, A. appendiculatus, and A. brachyurus from tubificids in Russia. She also found A. appendiculatus in the cyprinid fishes T. tinca, Abramis sapa (Pallas), A. brama, B. barbus, Cyprinus carpio (L.), Aspius aspius (L.), and Leucisus leucisus (L.).

Calentine and Ulmer, in an abstract (1961b), followed by a complete description by Calentine (1962), reported gravid specimens of a caryophyllaeid species, *Archigetes iowensis*, from oligochaetes (*L. hoffmeisteri*) and from fish (*Cyprinus carpio*) in Iowa. Although these cestodes also conformed closely to the generic diagnosis of *Biacetabulum*, they were assigned to the genus *Archigetes*, which was held to have priority over *Biacetabulum*, if a synonomy does exist.

Thus, at least several species of these two genera have been reported from fish or oligochaete hosts, but no unanimity of opinion regarding their taxonomic status has thus far been realized. Despite numerous published accounts on the morphology of individual species, experimental studies have been recorded for only four caryophyllaeid species. Although a complete life cycle involving both oligochaete and fish hosts has not been experimentally demonstrated for any caryophyllaeid cestode, it is generally believed that a single intermediate host is involved. Wisniewski's (1930) experiments with *A. sieboldi* have been mentioned previously.

Although larvae of *Caryophyllaeus laticeps* have long been known from naturally infected oligochaetes, the complete life history of this cestode has not been followed experimentally. Mrázek (1901) found that procercoids of this species, when 5 mm. long, may mature within tubificids. Sekutowicz (1934), reporting on the life cycle of *C. laticeps*, based his conclusions upon study of larvae from naturally infected oligochaetes (*T. tubifex* and *T. barbatus* Grube) and adults from naturally infected fishes (*Cyprinus carpio*, *A. brama*, and *Blicca bjorkna* L.). He found that larvae of *C. laticeps* (becoming mature, but never gravid) attained a maximum length of 20 mm in the annelid host. However, Dogiel et al. (1961) stressed that C. laticeps does not parasitize C. carpio, but occurs only in cyprinid fishes of the genus Abramis. They stated that Caryophyllaeus fimbriceps Chlopina, 1919, is the caryophyllaeid found in fishes of the genus Cyprinus in Eurasia.

McCrae (1961), in an abstract, reported on the life cycles of Glaridacris oligorchis Haderlie, 1953, G. catostomi, and a third (undescribed) species. He found gravid adults of these species in naturally infected fish (Catostomus commersoni) and larvae in naturally infected oligochaetes (L. udekemianus). As a result of experimental studies, McCrae found that procercoids of these three caryophyllaeids developed in L. udekemianus but not in L. hoffmeisteri or in T. tubifex. These procercoids develop a scolex typical of the adult tapeworm, but only primordia of gonads are produced within tubificid hosts. He did not experimentally infect the definitive fish host with these procercoids.

It is apparent that additional experimental studies are necessary in order to ascertain the taxonomic relationships of these cestodes. Since A. iowensis was available in quantity from Iowa River oligochaetes and fish, an experimental life history study was undertaken with this species. This report is a result of that study.

MATERIALS AND METHODS

Fishes were obtained with the use of hoop nets, seines and electric shocking equipment. Fish were usually examined, and cestodes preserved, in the field. Oligochaetes were collected by washing mud through a 40-mesh screen; approximately 1,000 tubificids were examined for the presence of helminths each month during the study.

Annelids parasitized by larval caryophyllaeids may be identified by the appearance of a conspicuous whitish region of the body. Infected oligochaetes were examined, under coverslip pressure, with the use of a 6X objective of a dissecting microscope. *Archigetes* is generally quite distinguishable from other larval caryophyllaeids because of its location within the seminal vesicles of the host, its distinctive scolex, and by the presence of gonads.

The excretory system of living worms was studied with the use of the 43X and 97X objectives of a compound microscope. Neutral red and Nile blue sulfate were added to some preparations, but details could be best studied with the use of tap or distilled water alone.

Oligochaetes were preserved in Bouin's fluid; cestodes removed from annelid and fish hosts were fixed in AFA. All whole mounts were stained in Mayer's paracarmine with fast green counterstain. Sections were stained in Harris's hematoxylin followed by triosin counterstain, or with Heidenhain's azan triple stain. After dehydration in ethanol, sections were cleared in xylene and whole mounts were cleared in xylene or methyl salicylate. Resinous media were used for all preparations. Drawings were made with the use of a microprojector or the camera lucida.

Techniques of egg recovery differed depending upon the host. Eggs of cestodes from carp were obtained by placing parasites in distilled water for several hours. Refrigeration during this time increased the number of eggs shed. Additional eggs were often obtained by tearing apart the bodies of such cestodes. Eggs of A. iowensis, deposited within the seminal vesicles of tubificids were collected by first removing the cestodes (still enclosed in the tissues of the vesicle) from the host. Cestodes were then placed in stender dishes and host tissue torn away, thus freeing the eggs. Those eggs retained within the bodies of parasites from tubificids were collected by dissection.

Eggs were washed to remove debris and then maintained in stender dishes at room temperature. Depending upon the number of eggs available at any one time, 1.5, 10, 30, or 45 ml containers were used. Stream water used in all egg cultures was filtered prior to use; culture water was changed daily. For purposes of study, egg samples were transferred to a glass slide, covered with a coverslip and examined with the use of a compound microscope.

Feeding experiments involving embryonated eggs and tubificids were usually conducted with the use of a mud medium. The mud used in all laboratory work was obtained from streams, screened with a 40-mesh sieve, and air-dried prior to use. Experiments were also conducted without the use of mud, but results were never as satisfactory as with its use. Approximately 500 to 800 embryonated eggs of A. iowensis from carp were added to about 10 cc of mud in a 100 ml glass container. Seventy to 100 tubificids were added and maintained at room temperature under continuous aeration. Following exposure to eggs, an interval varying from less than one hour to a maximum of three days in different experiments, tubificids from each experiment were removed and maintained in a separate container. Occasionally, a second group of oligochaetes was added to the container following removal of the first worms.

Tubificids were exposed to embryonated eggs of A. iowensis from oligochaete hosts in similar fashion. In this case, however, because of the much smaller number of eggs available at any one time (usually those from a single parasite), smaller containers, less mud, and fewer tubificids were used. Here, a 10 ml vial and three cc of mud were used to expose about 50 annelids to the eggs. Tubificids used in all experimental studies were either laboratory-reared specimens, or worms collected in nature and maintained in the laboratory three to six months prior to use. Such worms were examined for the presence of cestodes prior to use in feeding experiments. Tubificids used in these studies were fed a mixture of oatmeal, cornmeal and commercial goldfish food.

At frequent intervals, oligochaetes in the experiments were examined for the presence of cestode larvae. One or two worms were placed on a glass slide in a drop of water, covered with a coverslip and examined with a microscope. At first, a magnification of 100 diameters was needed to discern young larvae in the tubificid hosts, but after about 30 days, they could be distinguished with the use of a dissecting microscope.

Carp used in feeding experiments were taken from lakes or streams where A. iowensis had not been recovered from fish or tubificids. On one occasion, however, Iowa River carp were used for a specific experiment.

Other experiments involved white suckers as well as fathead and bluntnose minnows, all of which were taken from a small stream near Iowa Falls, Iowa. Several specimens of each species, examined soon after collection, harbored no cestodes. Goldfish used in a limited number of feeding experiments were obtained from commercial sources.

In feeding experiments involving A. iowensis and small fishes (2 to 6 inches in length) the latter were allowed to ingest infected tubificids. The fish were placed in individual, clean aquaria. Several infected oligochaetes were then placed on the bottom of each aquarium. After the fish had eaten the worms, the bottom contents of the aquaria were examined for the presence of regurgitated cestodes. Fish were examined at various intervals following feeding.

Larger fish (15 to 25 inches in total length) were force-fed. A bent glass tube, 7 mm in diameter and equipped with a length of rubber tubing at one end, was used on fish 12 to 20 inches in length. Infected oligochaetes and a small quantity of mud were drawn into the end of the tube by suction. While the fish was restrained with a wet burlap sack, the tube was forced into its stomach and the contents discharged. The fish was then placed in an aquarium for a time so that any regurgitated cestodes could be found.

Force-feeding of carp over 20 inches in length was accomplished with the use of a 15-inch length of aluminum tubing (10 mm in diameter) equipped with a plunger. The tube was bent slightly in order to conform to the curvature of the pharyngeal region of the carp. This feeding device was patterned after that described by Loeb and Kelly (1960).

Infected tubificids were either placed in a gelatin capsule or were confined between pieces of cotton at the end of the tube. The tube was forced into the stomach of the fish and the contents were discharged with the use of the plunger.

During the summer of 1961, carp were maintained in large cement tanks during feeding experiments. However, such tanks were not available for experiments conducted in 1962. Since digestive processes of fish appeared to be modified when the larger fish were maintained in small aquaria, a large wire container, submerged in the Skunk River (where *A. iowensis* had not been recovered from fish or from oligochaetes), was used to maintain carp during the experiments conducted in 1962.

SUMMARY OF LIFE CYCLE

Gravid specimens of *Archigetes iowensis* may occur within the intestinal tract of the cyprinid fish, *Cyprinus carpio*, or within the seminal vesicles of the tubificid oligochaete, *Limnodrilus hoffmeisteri*. Natural infections of annelids occur throughout the year, reaching a maximum in October. Carp infections are strictly seasonal, never occurring later than early July. Sexually mature fish may harbor more than 1500 cestodes, but immature carp normally harbor less than 100.

Eggs shed by parasites from fish are unembryonated; those from parasites of oligochaetes may or may not be developed. Oncosphere development requires 14 days in eggs derived from cestodes of fish. Under experimental conditions, *L. hoffmeisteri* becomes infected by ingesting embryonated eggs. After hatching in the tubificid's intestine, oncospheres penetrate the gut wall, usually between segments 17 to 22. Larvae migrate anteriad and enter the seminal vesicles of the host. A cercomer bearing six embryonic hooks, characteristic of the procercoid, appears between 25 to 32 days. By 40 to 50 days, the scolex is well-developed. Reproductive organs are formed by 70 days, at which time larvae average 1.0 mm in length. In nature, procercoids occasionally become ovigerous within tubificids and eggs may accumulate *in utero*, in the body parenchyma, in a cuticular sac surrounding the parasite, or occasionally may be released into the seminal vesicles of the host. Only those eggs retained within the body of the parasite or within a cuticular sac undergo embryonation. Larval development is similar, whether eggs of *A. iowensis* are derived from oligochaete or from fish hosts.

Although experimental feedings of *A. iowensis* to carp are not conclusive, fish apparently acquire infections by ingesting infected tubificids. Factors responsible for the periodicity of adults in carp are not thoroughly understood. However, evidence suggests that this periodicity may result from variations in the resistance of carp to cestode infection during the year.

GRAVID ADULTS

The definitive host of A. iowensis is the cyprinid fish, Cyprinus carpio. Although procercoids may produce eggs in the tubificid oligochaete, Limnodrilus hoffmeisteri, most parasites apparently require a fish host in order to become ovigerous. The morphology of gravid cestodes from both tubificid and fish hosts was presented in an earlier paper (Calentine, 1962). Cestodes from the two hosts differ in structure only in that specimens from oligochaetes (Figure 1) possess a cercomer, while those from fish (Figure 2) lack this appendage. The specific diagnosis (Calentine 1962, page 517) is repeated here.

> Archigetes iowensis. With the characters of the genus. Gravid adults averaging 1.6 mm (1.1-2.4) in length (without cercomer) by 0.52 mm (0.37-0.72) in width. Cestodes (from oligochaetes) possessing cercomer bearing 6 embryonic hooks. Scolex well-developed, bearing a distinct bothrium (accompanied by two loculi) on each of the dorsal and ventral surfaces. Terminal disc present. Frontal glands weakly developed. Neck cell complex present.
>
> Testes numbering 66 (57-76), medullary, 0.04 by 0.19 mm and extending from neck region to cirrus pouch. External seminal vesicle present, measuring 0.11 mm (0.09-0.12) by 0.08 mm (0.07-0.10). Cirrus pouch 0.15 mm (0.14-0.17) in diameter.
>
> Preovarian vitellaria located in two lateral bands. Vitelline follicles medullary, elliptical and measuring 0.03 by 0.08 mm. Follicles extending laterally along ovarian wings. Postovarian vitellaria present. Ovary dumbbell-shaped, entirely medullary. Ovarian wings 0.14 mm in length. Uterus uniting with vagina to form utero-vaginal canal, the latter opening with cirrus into a common genital pore. Seminal receptacle present. Uterus extending just anterior to cirrus pouch. Eggs (shed by cestodes from carp) measuring 32 by 47 microns.

In the previous account, it was stated that most gravid cestodes within oligochaetes deposit eggs inside the seminal vesicles. However, during 1962 it was found that a majority of gravid specimens in these hosts retained eggs *in utero* or within the body parenchyma (following rupture of the uterus). The reason for this apparent difference in findings is not known. One gravid cestode 1.5 mm in length was found to have 76 percent of the body filled with eggs (Figure 23). Wisniewski (1930) reported that in *A. sieboldi* as much as 66 percent of the body may be occupied by eggs. Displacement and degeneration of reproductive organs of *A. iowensis* accompanies the retention of eggs. Testes and vitellaria, the organs most often affected, may degenerate completely except in the region of the scolex. The ovary is often displaced ventrally, and in some cases undergoes partial atrophy. The cirrus pouch was never observed to be affected by the retention of eggs. Eggs of *A. iowensis* (within tubificids) may also accumulate between layers of cuticle in the region of the genital pore, but this condition was rarely seen.

GEOGRAPHICAL DISTRIBUTION

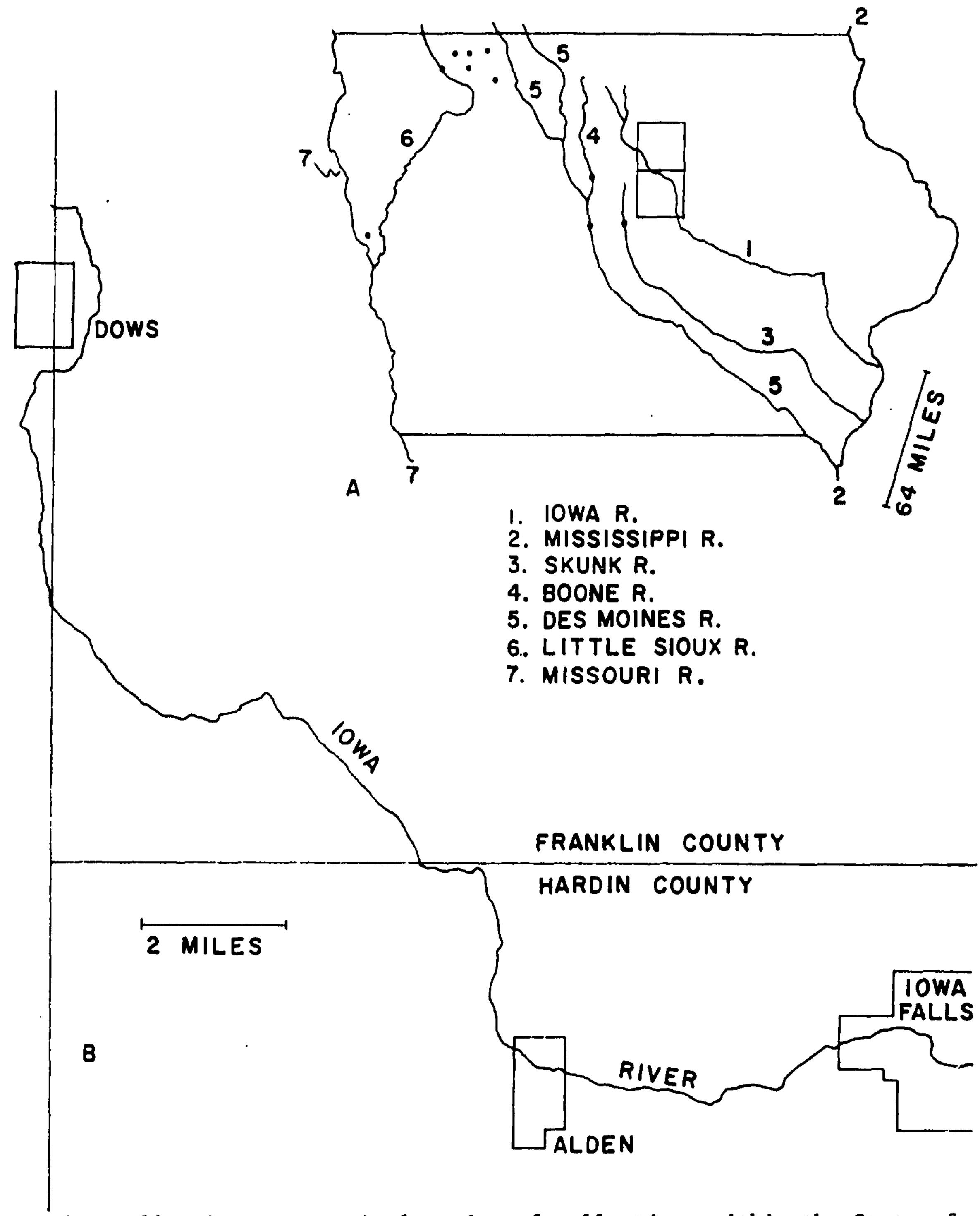
Carp from five rivers (Little Sioux, Des Moines, Boone, Skunk, and Iowa) in Iowa and from seven Iowa Lakes (East Okoboji, West Okoboji, Gar, Swan, Minnewashta, Blue, and Lost Island) were examined for the presence of helminths during those months when *A. iowensis* occurred in Iowa River carp. Surprisingly, *Archigetes* was found only in fish from the Iowa River, although another caryophyllaeid, *Khawia iowensis* Calentine and Ulmer, 1961, parasitized carp in all of these areas.

Since the Des Moines, Boone, Skunk, and Iowa Rivers are all associated with the Mississippi River drainage system, and since they offer similar ecological habitats with suitable tubificid hosts, one would normally expect carp in these waters to harbor similar parasites. *L. hoffmeisteri* occurs in at least some of the lakes mentioned above, and is very likely present in all of them. Although the survey of waters within the state is limited, the distribution of *A. iowensis* does appear to be more restricted than that of any other caryophyllaeid species yet found in Iowa.

The study area of the Iowa River comprised a 20-mile region between Dows and Iowa Falls, Iowa. In September, 1960, the area of river from just above Alden to below Iowa Falls was poisoned with rotenone by the Iowa State Conservation Commission. Hence, in 1961, carp were collected near Dows, above the poisoned area. *Ictiobus cyprinellus* (Val.), *Hypentelium nigricans* (LeSueur), *Moxostoma anisurum* (Raf.), *M. macrolepidotum* (LeSueur), and *M. erythrurum* (Raf.) have not been taken in collections made at the Iowa Falls impoundment since the rotenone poisoning. However, in 1962, carp were found to be abundant in both the Iowa Falls and Alden impoundments; fish collections were then made in both these areas in 1962.

Dams on the Iowa River, located at Iowa Falls and at Alden, Iowa, prevent movements of fish between these areas except for periods of spring flooding.

The distribution of fish collections within the state of Iowa, as well as those from the Iowa River, is indicated on Map 1.



Map 1. Collection areas. A: location of collections within the State of Iowa. Dots indicate collection sites. Franklin and Hardin Counties are enclosed by solid lines. B: the Iowa River study area

EGGS AND ONCOSPHERES

Studies were made on eggs of *A. iowensis* derived from three sources, namely those shed by cestodes from carp, those removed from the seminal vesicles of tubificids, and lastly eggs dissected from the bodies of parasites from tubificid hosts. Certain differences in size and degree of development characterize eggs from each of these sources.

Eggs shed by *A. iowensis* from carp are unembryonated and average 32 (30-34) by 47 (45-53) microns in size. The operculate egg (Figure 4), containing four to seven yolk cells, possesses a thin, smooth shell. Embryos are difficult to discern in freshly shed eggs, but in sections, zygotes of intrauterine eggs are readily visible and measure 11 microns in diameter. After five days in river water, the irregularly shaped embryos become distinctly visible (Figure 5). By the 10th day, they have become elongate and average 14 by 24 microns in size (Figure 6). Median hooks are now visible on some larvae. All hooks, directed toward the anopercular end, are present after 14 days. Growth of the embryo is now complete. The oncosphere (Figure 7) is elongate, measuring 15 (14-21) by 35 (32-42) microns. Lateral hooks average 7 microns in length, while the somewhat longer median hooks are 11 microns long. Flame cells could not be seen in living oncospheres. Two refractile cells are located near the median hooks. Wisniewski (1930) expressed belief that such cells are associated with the production of embryonic hooks. Living oncospheres of *A. iowensis* were maintained for 80 days at room temperature.

Eggs of *Archigetes*, released by cestodes and accumulating within the seminal vesicles of oligochaetes, are comparable in size to those of parasites of carp and measure 31 (28-35) by 46 (42-53) microns. Non-embryonated ones, removed from the seminal vesicles and placed in river water, were never observed to undergo development. Oncospheres were found only once within eggs inside the seminal vesicles. In this case, however, most eggs produced by the cestode were retained within the body parenchyma. The cuticular layer covering the genital pore had apparently ruptured and some embryonated eggs had escaped into the seminal vesicles. The presence of a gelatinous coating on eggs within the seminal vesicles of tubificids may account for the failure of these eggs to undergo development.

A larger size, 39 (38-42) by 67 (60-80) microns, distinguishes eggs retained within the voluminous uterus or within the body parenchyma of A. iowensis from tubificids. (Eggs of cestodes from carp were measured after 14 days in water, but no appreciable difference in size from freshly shed eggs was noted.) Eggs retained within the parasite undergo embryonation, and oncospheres are often present. Another characteristic feature of these eggs relates to their development, since most eggs of a single parasite are in similar stages of embryonation. Thus, if development has proceeded sufficiently, all viable eggs contain oncospheres. These oncospheres average 22 (21-24) by 53 (49-56) microns in size, and are larger than those of A. iowensis from carp hosts. The embryonation time of eggs inside the body of the parasite was not established. However, the development of these eggs, when removed from the body of the parasite and maintained in river water, approximates that for eggs of cestodes from carp. Intrauterine eggs, containing embryos comparable in size to those five days of age from *A. iowensis* derived from carp, were completely developed after ten days in water. Whether or not embryonation of eggs retained in the body of parasites proceeds at the same rate as it does in those placed in river water is not known.

Pronounced differences are apparent between *A. iowensis* and *A. sieboldi* with respect to egg size and time of development. According to Wisniewski (1930), eggs of *A. sieboldi* are always retained *in utero* and have a maximum size of 30 by 60 microns, being smaller than those eggs retained within the body of *A. iowensis*. Oncospheres of *A. sieboldi*, however, measure 22 by 51 microns, comparable to those of *A. iowensis*. In the former species, egg development is independent of temperature, requiring 40 days. Thus, the time of embryonation of this cestode is much greater than that of *A. iowensis*. Oncospheres of both species remain alive 70 to 80 days.

INTERMEDIATE HOSTS

General Account

Larval caryophyllaeids, known from naturally infected oligochaetes since 1855, have never been recorded from other aquatic invertebrates. Species of Archigetes, reported from Germany, Poland, Czechoslovakia, Brazil, Japan, Russia, and the United States, parasitize certain members of two tubificid genera, Limnodrilus and Tubifex. A. sieboldi has been reported from T. tubifex in Germany (Leuckart, 1878), from L. hoffmeisteri in Poland (Wisniewski, 1930), from L. udekemianus and L. hoffmeisteri in Brazil (Marcus, 1942), and from tubificids in Russia (Kulakowskaja, 1961). A. appendiculatus is found in L. claparedianus, T. tubifex, and L. hoffmeisteri in Czechoslovakia (Mrázek, 1897 and 1908), in oligochaetes in Russia (Kulakowskaja, 1961), and in L. gotoi Hatai, L. willeyi Nomura, and T. hatai Nomura in Japan (Motomura, 1929). (In a personal communication, Dr. Ralph Brinkhurst, University of Liverpool, expressed belief that L. gotoi Hatai is composed of L. hoffmeisteri in part and L. udekemianus in part, that L. willeyi Nomura is a synonym of L. udekemianus, and that T. hatai Nomura is a synonym of T. tubifex.) A. brachyurus is reported from L. hoffmeisteri in Czechoslovakia (Mrázek, 1908) and from tubificids in Russia (Kulakowskaja, 1961). A. cryptobothrius has been recorded only from L. hoffmeisteri in Poland (Wisniewski, 1928).

Five oligochaete species, namely L. hoffmeisteri, L. udekemianus, Tubifex tempeltoni Southern, 1909, Branchiura sowerbyi Beddard, 1892, and Dero limosa Leidy, 1880 were recovered from the Iowa River during the course of this investigation. *L. hoffmeisteri* and *T. tempeltoni*, found throughout the 20 miles of river under study, were most numerous in shallow, muddy areas where the current is negligible. The monthly tubificid collections described in the present account were made from this type of habitat. Here, *L. hoffmeisteri* comprised about 92 percent and *T. tempeltoni* about 7 percent of all annelids collected. Specimens of *L. udekemianus*, obtained only in limited numbers, were found almost exclusively in river areas of more rapid current and with sand or gravel bottom. *B. sowerbyi*, not abundant in any area, was restricted to regions of mud bottom, but the distribution of this species was very irregular.

Other invertebrates in the Iowa River were also examined for the presence of caryophyllaeid larvae. Several hundred chronomids and mayfly nymphs, the predominant invertebrates other than tubificids, were examined during both spring and fall. However, natural infections of caryophyllaeids were found only in oligochaetes.

In nature, caryophyllaeid cestodes of several species were found in *L. hoffmeisteri*, *L. udekemianus* and *T. tempeltoni*, but specimens of *A. iowensis* were recovered only from *L. hoffmeisteri*. However, because of the small number of *L. udekemianus* recovered, its role as a possible host for *A. iowensis* cannot be fully ascertained at this time. The relative scarcity of this tubificid species in the Iowa River indicates that its role in nature would be, at most, a minor one.

Although *L. hoffmeisteri*, *T. tempeltoni*, and *D. limosa* were later exposed to embryonated eggs of *A. iowensis* under experimental conditions, development occurred only in *L. hoffmeisteri*.

Location of Parasites

Descriptions of most species of Archigetes (from tubificids) are based upon specimens recovered from the body cavity of the host. Thus, A. cryptobothrius and A. brachyurus are reported only from this site in oligochaetes. A. sieboldi has been reported from the body cavity of tubificids (Leuckart, 1878 and Wisniewski, 1930), but Marcus (1942) found them within the seminal vesicles and ovisac of annelids. A. appendiculatus is found both within the body cavity and seminal vesicles of annelids (Mrázek, 1897 and Motomura, 1929).

All mature and gravid specimens of A. iowensis in naturally infected L. hoffmeisteri were present within the seminal vesicles (Figure 15). Annelids of this species possess two seminal vesicles (Figure 13). The anterior vesicle, confined to segment nine, is formed by a forward extension of the septal wall between segments nine and ten. The second vesicle, formed as a posterior pouching of the septal wall between segments ten and 11, varies in extent depending upon the age and state of sexual activity of the worm. In sexually active tubificids, the posterior vesicle may extend to segment 18. In immature and in sexually inactive worms, this vesicle usually extends posteriad for only one to three segments. The ovisac, formed as a posterior pouching of the septal wall between segments 11 and 12, encloses the posterior seminal vesicle, and in gravid specimens may extend to segment 21. Two spermathecae, becoming much enlarged when filled with spermatophores, are located in segment ten. Spermathecal apertures are present on the ventral surface of this segment. A single pair of testes is present on the anteroventral surface of this same segment. Each of the two male funnels, located at the posterior surface of segment ten, leads to a vas deferens which coils in segments 11 and 12. A conspicuous prostate surrounds a portion of each vas deferens. Each male duct terminates in a penis, surrounded by a distinct chitinous sheath. The male genital apertures are found on the ventral surface of segment 11. The paired ovaries, similar in position to the testes, are found in segment 11. Each oviduct, possessing a ciliated funnel, leads to a female pore on the ventral surface of segment 12. The seminal vesicles and ovisac, dorsal to the gut, are profusely supplied with blood vessels (Figure 14).

Most specimens of A. iowensis localize in the posterior vesicle, where as many as three (natural infections) or four (experimental infections) mature cestodes were found. In a single tubificid, as many as 18 immature larvae were recovered from this vesicle, 30 days post-infection. Cestodes in single infections are usually fully extended, with scoleces directed either toward the anterior or posterior of the host's body. Parasites in multiply infected hosts may be doubled upon themselves or may be coiled. Only a single mature tapeworm, usually coiled, was ever present in the anterior vesicle.

During examination of more than 200 experimentally infected oligochaetes in 1961, mature cestodes were found only once outside the seminal vesicles. In this tubificid (227 days post-infection) harboring four mature cestodes inside the posterior vesicle, two mature parasites were found within the body cavity between segments 34 to 43 and 45 to 53 respectively.

Wisniewski (1930) indicated that because of the intimate contact between specimens of A. sieboldi and annelid blood vessels, the latter adhere to parasites when removed from the host. Mrázek (1897, Figure 2) illustrated blood vessels adhering to A. appendiculatus following removal from oligochaetes. Blood vessels were also attached to many A. iowensis upon dissection from tubificids. However, it was found that these blood vessels were present only when tissues of the seminal vesicle and ovisac still enclosed the parasites. With the removal of these membranes, the blood vessels were also detached.

Mrázek found individuals of A. appendiculatus both within the body cavity and within the seminal vesicles of oligochaetes, which may account for the adherence of blood vessels to some cestodes. Wisniewski, however, stated that mature and gravid A. sieboldi were present only within the body cavity of the annelid host. Marcus (1942), on the other hand, reported this caryophyllaeid from the seminal vesicles and ovisac of tubificids. It would seem that at least some of the cestodes studied by Wisniewski were actually located within the seminal vesicles or ovisac.

Pathology

The presence of parasites within oligochaetes is responsible for several pathological conditions. Spermatozoa are usually lacking within the posterior seminal vesicle if mature cestodes are present. The anterior seminal vesicle of adult tubificids usually contains spermatozoa; these parasites seldom occur in this location. However, parasitized annelids rarely produce ova, even though their ovaries appear normal.

The growth of cestodes, localized within the posterior seminal vesicle of immature or of sexually inactive oligochaetes, results in tissue hyperplasia of the host so that these organs enlarge and extend posteriad with the growth of the tapeworms.

The body of parasitized annelids is often distended and weakened at the site of infection, especially by the presence of multiple infections in the posterior vesicle. A single cestode within the anterior vesicle often produces the same effect. The body wall may rupture, freeing the parasite. Compression of the tubificid's intestinal tract usually occurs; complete obstruction was observed in one specimen.

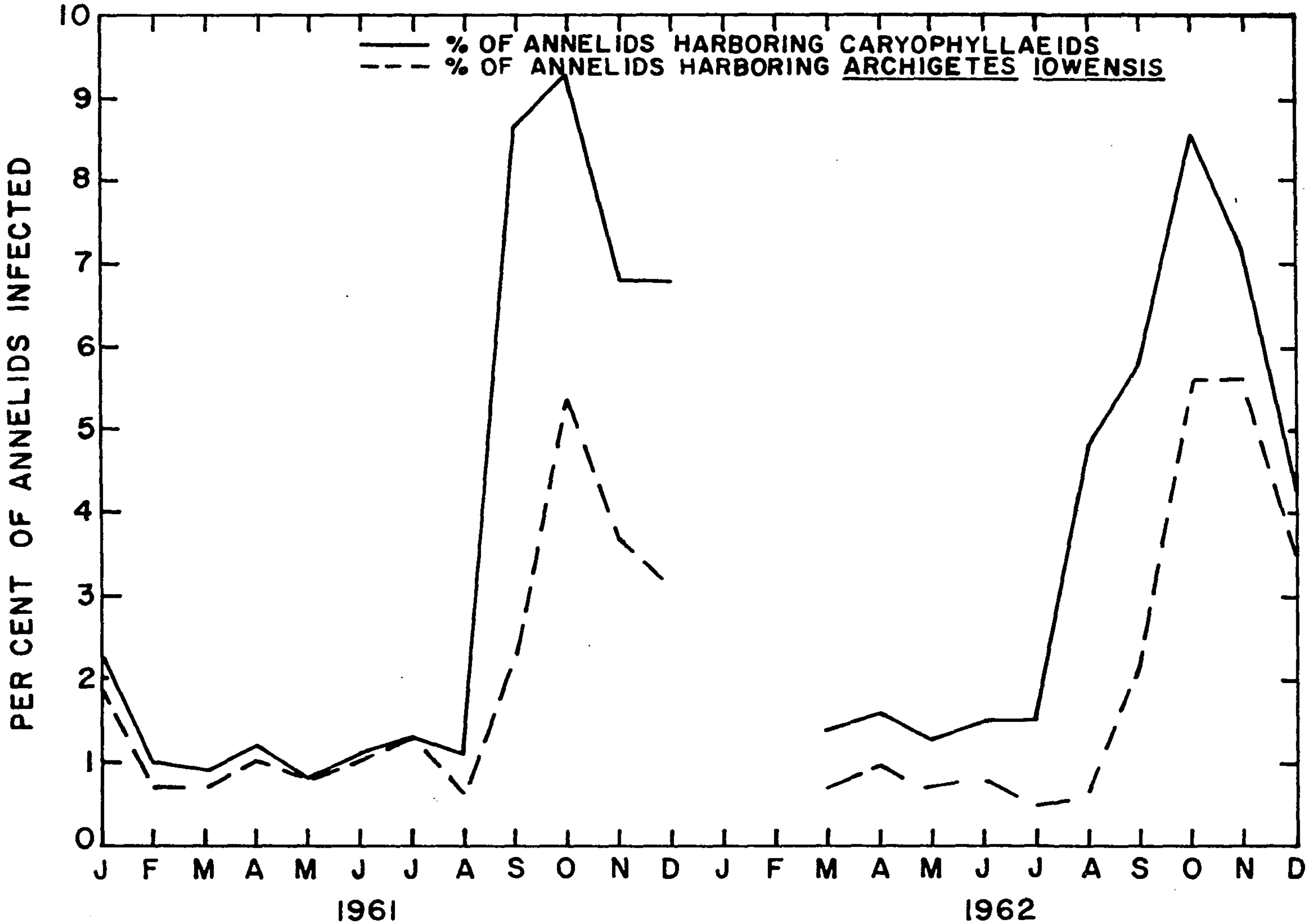
Death of annelid hosts was noted during experimental studies. As many as 18 procercoids were found in a single oligochaete, 30 days post-infection. However, tubificids harboring more than 11 larvae survived no longer than 125 days, those infected with seven to ten procercoids lived at most 157 days and annelids harboring five to six cestodes perished before 259 days. No more than four mature *A. iowensis* were present in experimentally infected tubificids after 365 days.

Natural Infections

During examination of 26,000 *L. hoffmeisteri* collected from the Iowa River during 1961 and 1962, 948 (3.7 percent) harbored caryophyllaeids. *Archigetes iowensis* occurred in 546 (58 percent) of the infected tubificids. Thus, 2.1 percent of all *L. hoffmeisteri* examined were infected with *Archigetes*. Additional infected oligochaetes were collected during these two years (without noting the degree of parasitism) so that more than 1300 specimens of *A. iowensis* were recovered. *L. hoffmeisteri* harbors larvae of several caryophyllaeid species; procercoids of two or more species may parasitize a single annelid. A maximum of three *Archigetes* was found within one tubificid; seven percent of oligochaetes infected with *A. iowensis* harbored multiple infections. Specimens of *L. hoffmeisteri*, as small as 8 mm in length, often harbored mature *Archigetes*. The percentage infection of Iowa River *L. hoffmeisteri* with caryophyllaeid cestodes during the interval of January, 1961 through December, 1962, is shown in Graph 1. Collection details are given in Table 1.

It is of interest that infections of *A. iowensis* in carp show a pronounced peak, and that there is also a seasonal abundance of larvae in tubificids. Oligochaetes apparently acquire their infections of *Archigetes* prior to October, as shown by data from natural collections and from experimental studies. Collection records indicate that gravid adults in fish do not appear after early July (Graph 2), and experimental studies show that oncospheres live for a maximum of 80 days. Thus, maximum infection of annelids occurs during October.

Although some immature larvae are present in tubificids throughout the year, they predominate in late summer and early fall. When immature larvae are found at other times during the year, they usually occur as multiple infections. Very young specimens of *A. iowensis* cannot be identified with certainty since procercoids of several other caryophyllaeid species are also found in *L. hoffmeisteri*. Larvae of two genera, other than *Archigetes*, localize within the seminal vesicles and cannot



Graph 1. Infection of Iowa River Limnodrilus hoffmeisteri with caryophyllaeid cestodes during 1961 and 1962

Table 1. Details of tubificid (*Limnodrilus hoffmeisteri*) collections from the Iowa River during 1961 and 1962

	Number of *L. hoffmeisteri* examined	Number infected with	
		Caryophyllaeid larvae	*A. iowensis*
1961			
January	785	18 (2.3%)	15 (1.9%)
February	1165	11 (1.0%)	8 (0.7%)
March	1154	10 (0.9%)	8 (0.7%)
April	1494	18 (1.2%)	15 (1.0%)
May	1187	10 (0.8%)	10 (0.8%)
June	1144	13 (1.1%)	12 (1.0%)
July	1284	17 (1.3%)	17 (1.3%)
August	1014	11 (1.1%)	6 (0.6%)
September	953	82 (8.6%)	21 (2.2%)
October	1370	127 (9.3%)	74 (5.4%)
November	1054	72 (6.8%)	39 (3.7%)
December	973	66 (6.8%)	31 (3.2%)
1962			
January	0[a]		
February	0[a]		
March	1154	16 (1.4%)	8 (0.7%)
April	1194	19 (1.6%)	12 (1.0%)
May	1310	17 (1.3%	9 (0.7%)
June	1197	18 (1.5%)	10 (0.8%)
July	1034	16 (1.5%)	5 (0.5%)
August	1029	49 (4.8%)	6 (0.6%)
September	1222	71 (5.8%)	27 (2.2%)
October	1210	104 (8.6%)	68 (5.6%)
November	1800	129 (7.1%)	100 (5.6%)
December	1273	54 (4.2%)	45 (3.5%)
Totals	26000	948 (3.7%)	546 (2.1%)

[a]No collections made; collection area completely frozen.

be differentiated from the latter until scoleces have developed.

The abrupt increase in the infection curve (Graph 1) between August and September, 1961 and between July and August, 1962 indicates that some oligochaetes harboring immature larvae were probably overlooked during examination. During the months July through September, many larvae in tubificids are too young for specific identification; the percentage infection with A. iowensis is probably greater than indicated. During study of experimentally infected annelids, it was found that parasitized individuals could usually be distinguished with the naked eye 40 days post-infection. A. iowensis can be differentiated from other procercoids by 50 days.

Infections of Archigetes in tubificids, after reaching a peak (5.6 percent) in October, gradually decrease until February. From this time until July or August, approximately one percent of these annelids are parasitized.

Gravid A. iowensis, although occasionally recovered from oligochaetes, seem to be limited in these hosts to the interval March through August. During these months in 1962, 567 mature cestodes were collected, but only 18 (3.2 percent) were ovigerous.

Natural infections of oligochaetes with A. iowensis and A. sieboldi are similar in that the same host (L. hoffmeisteri) may be infected and that both species exhibit seasonal periodicity. Differences between the two species occur in the percentage of oligochaetes infected, in the maximum number of cestodes harbored by a single host, and in certain aspects of periodicity. During examination of 4,000 L. hoffmeisteri in

Poland, Wisniewski (1930) found 340 (8.5 percent) naturally infected with *A. sieboldi*, a degree of infection greater than that in *A. iowensis* (2.1 percent). Wisniewski found that 49 percent of infected annelids harbored more than one parasite; a maximum of eight cestodes was recovered from a single tubificid. In the current study, only seven percent of infected annelids harbored more than one specimen of *A. iowensis*, with a maximum of three mature worms found in an individual oligochaete. Although a seasonal periodicity characterizes both species, certain differences are notable. Wisniewski found immature larvae to be most abundant in October, but mature procercoids did not attain a definite peak. He found that gravid *A. sieboldi* reached a well-defined peak in July; more than 60 percent of all specimens recovered from oligochaetes during this month were gravid. Infections of tubificids with mature larvae of *A. iowensis* attain a maximum in October, but gravid cestodes never comprise a significant portion of the population.

Experimental Studies

Experimental studies on *A. iowensis* were conducted with eggs derived from two sources: those obtained from gravid worms in carp and those dissected from parasites of tubificids.

Eggs of *A. iowensis* from carp

Because gravid specimens of *A. iowensis* are most numerous in carp during the month of May, eggs were collected and experimental studies were undertaken at this time in 1961 and 1962. During 1961, eggs were obtained by placing gravid parasites in distilled water for one to six hours. In 1962, eggs were collected in similar fashion, and also by dissecting them from the bodies of tapeworms. Eggs were maintained at room temperature in river water for 18 to 25 days prior to use in experiments. Feeding experiments were conducted by mixing embryonated eggs with a small quantity of mud, to which tubificids were then added. Some experiments involved the exposure of a single oligochaete species to the eggs; in others, two or three species of annelids were exposed in a common container.

The oligochaetes *Limnodrilus hoffmeisteri*, *Tubifex tempeltoni*, and *Dero limosa* were exposed to embryonated eggs of *A. iowensis*. Infections occurred, however, only in *L. hoffmeisteri*.

Dero limosa, a small species of the family Naididae, averages about 5 mm in length and may be too small to ingest eggs of *A. iowensis*. No species of *Archigetes* have been reported from oligochaetes of this family. *T. tempeltoni*, a larger species than *D. limosa*, but smaller than *L. hoffmeisteri*, did not acquire infections with *A. iowensis*, nor were natural infections ever found. That this oligochaete, however, is capable of ingesting eggs larger than those of *A. iowensis* was experimentally demonstrated. In the course of 14 experiments, *T. tempeltoni* in all instances readily ingested eggs and harbored infections of two other caryophyllaeid species, including *Glaridacris catostomi*. The eggs of this caryophyllaeid measure 42 by 60 microns, being larger than those of *A. iowensis* from carp. On the basis of this evidence it is concluded that *A. iowensis* does not develop in *T. tempeltoni*.

In nature, tubificids probably acquire their infections of *A. iowensis* while very young. This was shown to occur during experimental studies, since small, sexually immature specimens of *L. hoffmeisteri* (those approximately one inch in length) became more readily infected than did larger specimens. Furthermore, very few gravid tubificids acquired infections.

Results of four feeding experiments conducted in 1961 with *L. hoffmeisteri* are summarized in Table 2. It should be noted that tubificids in experiments three and four had a significantly higher rate of infection than those in the first two. Since the experiments differed only in the ages of the embryonated eggs at the time of exposure, it is probable that oncospheres require more than 18 days to become infective.

In the 1961 experiments, oligochaetes were exposed to embryonated eggs for three days; tubificids from individual experiments were then removed and maintained in separate containers. Ages given for these larvae are based upon the second day of exposure and have a maximum error of one day. Additional experiments conducted in 1962 involved exposure of tubificids to embryonated eggs for periods varying from less than one hour to three days.

Although hatching of the oncosphere and its penetration of the oligochaete's intestinal wall were not observed, oncospheres were seen within the body cavity of annelids less than one hour post-exposure. Study of these infected oligochaetes at this time indicates that penetration occurs between segments 15 to 28. Of six infected tubificids (one to six hours after exposure to eggs), harboring a total of 15 embryos

Table 2. Summary of four feeding experiments conducted with Limnodrilus hoffmeisteri and embryonated eggs of Archigetes iowensis (from carp) in 1961. Tubificids in each experiment were exposed to embryonated eggs for three days, then removed and maintained in separate containers

	Experiment number			
	1	2	3	4
Number of tubificids exposed	78	99	86	72
Egg incubation time, in days, prior to exposure	18	18	22	22
Number of tubificids infected 32-35 days post-feeding	24 (31%)	36 (36%)	50 (58%)	45 (63%)
Number of tubificids harboring multiple infections	13 (17%)	20 (20%)	36 (42%)	35 (49%)
Maximum number of Archigetes per host 32-35 days post-feeding	--	11	11	18
Duration of infection, in days	72	560	560[a]	560[a]

[a]Experiments not yet terminated.

within their coelomic cavities, larvae localized in that area of the body between segments 15 to 28. Most larvae, however, were situated between segments 17 to 22. At six days post-feeding, eight infected oligochaetes were examined. Ten larvae in the body cavities of these tubificids were situated between segments 11 to 25, with most of them localized between segments 12 to 15. At 15 days, 14 embryos in nine hosts were all located between segments nine to 13; most were found between 11 to 13. At this time, some cestodes were within the seminal vesicles, and by 20 days, practically all were present in this location.

Larvae migrate to segment ten (into which segment both vesicles open), then enter the seminal vesicles. There is no indication of entrance by direct penetration through the walls of the vesicles. The posterior vesicle harbors more parasites than does the anterior; procercoids are only occasionally present in the latter vesicle. Reasons for this apparent preference are unknown.

Embryos move incessantly until they enter the seminal vesicles; once within the vesicles, larval movement becomes much reduced. During movements, the hooks are directed posteriad.

During the first 30 days within tubificids, procercoids are oval to ovoid. The cercomer appears early in development, and is evident on living larvae at 25 days, but in fixed preparations it is usually not distinct until about 32 days. The cercomer is now little more than a constriction at the posterior of the body. (Figure 8). It then becomes progressively longer and thinner, and by 40 days it may attain a length approximately equal to one-third that of the body. In gravid specimens, from naturally infected hosts, the cercomer length varies between 0.3 and 0.7 mm (one-third to more than one-half the body length).

Procercoids often differ in size and degree of maturation after 30 days. The following description in regard to larval development and growth is based upon those of singly infected hosts where less variation occurs.

At 30 days post-infection, procercoids are oval with a length-to-width ratio of approximately three to two. By 40 days, they have become distinctly elongate and possess a length-to-width ratio of two (or more) to one. Formation of the dorsal and ventral bothria takes place between 35 and 40 days; general shape of the scolex is often discernible by 40 days. Minimal and maximal larval development at 40 days (in singly infected hosts) are shown in Figures 9 and 10. Although nuclear aggregates may now occur in the region of the testes, no organs are distinguishable in the conspicuous nuclear mass at the posterior of the body.

By 50 days, scolex formation is nearly complete (Figure 11). Although excretory ducts are never present at 40 days, morphological differentiation of this system is complete by 50 days. Individual testes are now well defined; the cirrus pouch and seminal vesicle are distinct. Primordia of vitelline follicles may appear lateral to the testes. The ovary is distinguishable on some specimens, and after 60 days is always present (Figure 12). By 70 days, all gonads and their associated ducts are fully formed.

Little growth of procercoids occurs until they enter the seminal vesicles. Maximum growth takes place between 15 and 50 days, following which, further increase in size occurs slowly. Length of larvae at ten-day intervals is presented in Table 3. Cestodes in singly infected hosts attain minimal definitive size (1.05 mm) by 100 days, but those in multiple infections consistently vary greatly in size and degree of maturation. For example, one infected annelid, 118 days post-infection, contained 11 larvae ranging in length from 0.18 to 0.95 mm. The largest specimen was mature, but the degree of maturation of the smaller cestodes in this host was comparable to those 32 days of age in single infections. Since the

Table 3. Growth of Archigetes iowensis procercoids, experimentally reared in Limnodrilus hoffmeisteri. Measurements, expressed in millimeters, were made from fixed, unflattened whole mounts and sections. Cercomer length is not considered. Those worms ten to 90 days of age were taken from singly infected hosts of one experiment

Age, in days	Average length	Range
0 (oncosphere)	0.04	0.03-0.04
10	0.06	0.05-0.08
20	0.13	0.09-0.15
30	0.28	0.18-0.41
40	0.66	0.24-0.98
50	0.89	0.80-0.98
60	0.93	0.75-1.10
70	1.01	0.92-1.10
80	1.07	0.95-1.23
90	1.11	0.98-1.20
460	1.40	1.38-1.43
Gravid cestodes from naturally infected hosts	1.53	1.05-2.30

largest A. iowensis recovered from a naturally infected annelid was 2.30 mm in length, it is apparent that considerable growth may occur post-maturation. It is of interest to note that experimentally reared cestodes 460 days of age are not so large as average-sized gravid worms in nature.

Although procercoids are mature by 100 days post-feeding, experimentally reared A. iowensis over 560 days of age are currently being maintained in tubificid hosts, but no procercoids have yet produced eggs.

Eggs of A. iowensis from tubificids

Embryonated eggs (retained with the voluminous uterus, within the body parenchyma, or deposited between layers of cuticle) of cestodes from tubificids are infective to L. hoffmeisteri. Tubificids in five of six feeding experiments conducted with such eggs became infected.

Information concerning the development of these larvae is less complete than that for procercoids derived from eggs of Archigetes from fish. Most of these experiments involved the exposure of those eggs recovered from the body of a single cestode; only a few annelids became infected in each experiment. Development of these procercoids is similar to that described for embryos derived from eggs of cestodes from fish. However, more variation occurred in the rate of procercoid development in the tubificid-to-tubificid infections. In the latter experiments, some procercoids were observed to mature by 70 days, but in others, development was much retarded. Two procercoids were maintained for more than 100 days; although one became ovigerous by 100 days, the other produced no eggs by 187 days.

Larval development of A. iowensis is strikingly similar to that of A. sieboldi. Wisniewski (1930) conducted experimental studies on the latter species by exposing gravid cestodes (from tubificid hosts) to specimens of L. hoffmeisteri. He established seven experiments, with four to 15 oligochaetes in each experiment. Eleven to 47 percent of the tubificids in six of the seven experiments became infected. Although

Wisniewski did not observe penetration of the tubificid's intestinal wall by the oncosphere, he found young larvae distributed throughout the coelomic cavity, and as far posteriad as segment 98. Procercoids were observed to then migrate to the gonadal region of the oligochaete, but according to Wisniewski, only young forms were ever found within the seminal vesicles. He observed a seasonal variation in the rate of development, with procercoids undergoing more rapid development in summer than in winter. Thus, in summer, larvae of *A. sieboldi* required 60 to 70 days to attain maturity and 100 to 110 days to become gravid. In winter, 160 to 170 days were needed for maturation and 200 to 210 days elapsed before egg production.

Although the time necessary for maturation is identical in the two species (60 to 70 days at summer temperatures), egg production in *A. sieboldi* appears to be much more prevalent than in *A. iowensis*.

Progenetic Development

Although progenetic development is described for all species assigned to the genus *Archigetes*, the extent of its occurrence within a species has been reported only for *A. sieboldi* (by Wisniewski in 1930) and for *A. iowensis* (the present study). Wisniewski found that procercoids of *A. sieboldi* commonly produce eggs in annelid hosts; gravid larvae became most abundant in July, at which time 60 percent of all cestodes recovered from oligochaetes were ovigerous. He showed experimentally that such eggs were infective.

Although Szidat (1937) believed he had found *A. sieboldi* in fish hosts, Janiszewska later (1950) indicated that Szidat's specimens represented A. appendiculatus. Thus, A. sieboldi is apparently known only from tubificids.

Results of field collections and of experimental studies involving A. iowensis show that progenesis is not common in this species. In nature, gravid procercoids are found in tubificids during the months of March through August, but only 18 (3.2 percent) of 567 parasites recovered from these hosts in 1962 were gravid. Experimentally reared procercoids (eggs derived from parasites of carp) were maintained in the laboratory for more than 560 days at room temperature, but none ever became ovigerous. Indeed, many larvae were observed to undergo degeneration and death between 365 and 560 days. Spermatozoa were commonly observed in mature procercoids although no eggs were present. Since the genital pore is usually covered by cuticle, and since gravid procercoids (when they occur in nature) are present only in singly infected annelids, it is evident that self-fertilization does occur in these worms.

The fact that one procercoid of A. iowensis (reared from eggs of cestodes from tubificids) became ovigerous by 100 days post-infection, while none of the procercoids (derived from eggs of cestodes from carp) became gravid by 560 days, indicates that the source of the eggs may be responsible for this difference. Perhaps only those procercoids, developing from eggs of A. iowensis derived from oligochaetes, are capable of becoming ovigerous in annelid hosts. The low percentage of gravid procercoids in nature may also be a result of this factor. Because of the surprisingly low incidence of progenesis in relation to the high percentage infection of oligochaetes and fish, transmission of infection in nature must be largely dependent upon fish hosts. Additional study regarding the occurrence of progenesis in *A. iowensis* is needed, especially with respect to the factors initiating egg production by procercoids.

DEFINITIVE HOSTS

General Account

Although members of the genus Archigetes have been known from tubificids since 1878, they were not associated with fish hosts until 1937. Szidat (1937) found caryophyllaeid parasites in the cyprinid fish Tinca tinca, and considered them to be adults of A. sieboldi. Janiszewska (1950) found similar cestodes in the fishes T. tinca, Barbus barbus and Abramis brama. However, Janiszewska believed her specimens, as well as those described by Szidat in 1937, to be adults of A. appendiculatus, not A. sieboldi. It should be noted that both Szidat and Janiszewska, in the above-cited publications, then transferred the species of Archigetes under consideration to the genus Biacetabulum Hunter, 1927. Comparison of Polish specimens labeled B. appendiculatum with Szidat's (1937) illustration and description indicates that the species represented is probably appendiculatus.

Kulakowskaja later (1961) reported A. appendiculatus (=B. appendiculatum) from cyprinid fishes in Russia. A. iowensis also parasitizes a cyprinid fish, Cyprinus carpio, but A. cryptobothrius, A. brachyurus, and apparently A. sieboldi are known only from oligochaete hosts.

Ten species of catostomid and ten species of cyprinid fishes were present in the Iowa River study area at the time of this investigation. A total of 902 fishes, including representatives of all these species, were examined for the presence of helminths during 1961 and 1962. A. iowensis was recovered only from the cyprinid fishes carp (Cyprinus carpio

L.) and northern common shiners, *Notropis cornutus* (Mitchill). In 1962, 12 *N. cornutus*, collected in April and May were dissected. Three of these each harbored a single mature *A. iowensis*, but no gravid adults were found. This fish apparently constitutes an accidental host for *Archigetes*.

Fishes examined and percentage infection with *A. iowensis* and other caryophyllaeids are listed in Table 4. Since many of the cestodes recovered during this study have not been described, names are not given to them. Details of natural infections of Iowa River carp with *A. iowensis* are presented in a separate section.

Location of Parasites

Specimens of *A. iowensis* are found in the stomach and throughout the entire length of the carp intestine. In fish examined immediately following capture, those cestodes present in the stomach and first third of the intestine are normally attached to the gut mucosa (Figure 29), while those in the last two-thirds of the tract usually lie free in the intestinal contents.

These cestodes probably remain within the fish host for a relatively short time. During field examination of carp, those fish having intestines well-filled with food were most likely to harbor large numbers of parasites.

Table 4. Infection of Iowa River catostomid and cyprinid fishes with *A. iowensis* and other caryophyllaeids during 1961 and 1962

	Number examined	Number infected with	
		A. iowensis	Other caryophyllaeids
Catostomidae			
Carpiodes carpio	24	0	11 (46%)
C. cyprinus	6	0	3 (50%)
C. forbesi	11	0	4 (36%)
C. velifer	5	0	4 (80%)
Catostomus commersoni	353	0	242 (69%)
Hypentelium nigricans	37	0	15 (41%)
Ictiobus cyprinellus	33	0	0
Moxostoma anisurum	4	0	1 (25%)
M. erythrurum	30	0	7 (23%)
M. macrolepidotum	53	0	22 (42%)
Cyprinidae			
Cyprinus carpio	261	98 (39%)	176 (67%)
Hybopsis biguttata	7	0	0
Notropis cornutus	12	3 (25%)	0
N. dorsalis	8	0	0
N. spilopterus	7	0	0
N. stramineus	9	0	0
N. topeka	10	0	0
Pimephales notatus	12	0	0
P. promelas	10	0	0
Semotilus atromaculatus	10	0	0
Total	902		

Pathology

In contrast to the well-defined pathology caused by procercoids of A. iowensis in tubificids, adults appear to produce little detrimental effect on the definitive fish host, even though carp may harbor more than 1500 cestodes. Harmful effects on the fish are probably limited because these cestodes: (1) are very small, (2) evidently remain in fish only a short time, (3) often occur free in the gut contents, and (4) do not usually damage the gut epithelium when they are attached to the host.

Those specimens of Archigetes attached to the lining of the intestinal tract may adhere very superficially, or may be almost completely buried in the mucosa. Study of sections indicates that attached worms localize in the crypts of the mucosa (Figure 29). The mucosal epithelium is usually intact, although it may be highly compressed in certain regions. Small areas of epithelium may be denuded, especially where host tissue is drawn into the bothria of the parasite. No inflammatory reaction on the part of the host was ever observed.

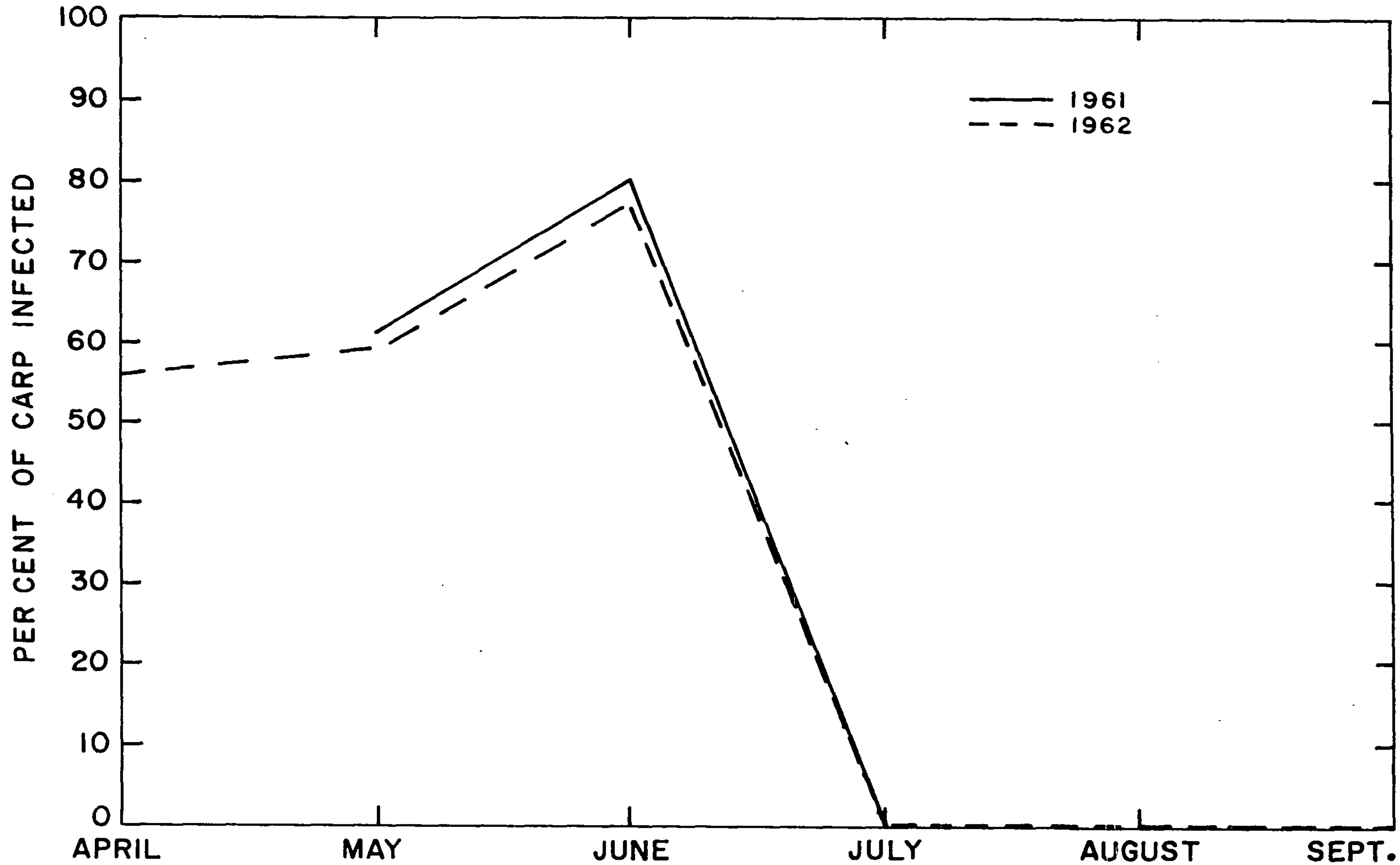
Natural Infections

In 1961, carp collections were made during the months of May through November (with the exception of August) and in 1962, they were made each month from April through November. No data are available for the months of December through March; collecting at this time is extremely difficult because of the heavy ice cover. Since the 1961 collections are not as complete as those of 1962, the following details concerning A. iowensis are based upon studies conducted during the latter year, unless otherwise specified. Eleven carp collections were made during the months of April, May and June. In addition, two were made in July and one each in the months of August through November.

One of the striking characteristics of _A. iowensis_ is its seasonal distribution in carp; this periodicity occurred during both years of field studies (Graph 2). In the spring of the year, percentage infection of carp may be as high as 80 percent, with more than a thousand tapeworms within a single fish. On the other hand, no infected carp were ever recovered during the months of July, August, and September of either 1961 or 1962. Although some _Archigetes_ were recovered from carp during October and November of 1962 (data not shown on Graph 2), evidence indicates that these do not represent normal infections.

The first fish collection in 1962 was made on April 6, less than two weeks after the ice cover left the river. It is of interest that at this time carp already harbored infections of _A. iowensis_, although all fish examined the previous November were negative. On April 6, 36 carp were examined; 19 (53 percent) were parasitized, with an average of three (1-41) cestodes per infected fish. Comparison with later findings for May and June, 1962, shows that this constitutes a light infection.

Infected carp were recovered during every collection in April, May, and June, including that of June 30, 1962. However, all of the 14 adult and immature carp examined on the succeeding collection (July 11) were negative for _Archigetes_. Although 78 carp (total fish for both years) were examined in the months of July, August, and September, none were infected. In 1961, infected fish were present no later than June, but in



Graph 2. Percentage infection of carp with <u>Archigetes iowensis</u> during the months of April through September of 1961 and 1962. See text for details of October and November collections

1962, some carp were found to harbor Archigetes in October and November. In October, one cestode was found in each of two carp, while a third harbored 109 specimens. In November, three of six carp examined were infected, harboring a total of 34 parasites. Fish infections at this time of year, however, differ significantly from those in the spring. For example, 123 of the 145 A. iowensis recovered during these months were highly deformed. Scoleces of these cestodes are usually misshapen and are often completely lacking; their bodies are often conspicuously swollen. Since the fish harboring these deformed parasites were examined in the field immediately upon collection, and since the cestodes were fixed as soon as removed, such deformities cannot be associated with techniques of handling. In addition, none of the cestodes were attached to the intestinal mucosa, and although all were mature, none were gravid.

During the interval that normal infections prevailed in 1962 (April 6 through June 30), 130 carp were examined; 77 (59 percent) harbored A. iowensis. Percentage infection of carp was greatest in June of both years (80 percent in 1961 and 77 percent in 1962). However, the average number of cestodes per infected fish was at a maximum in May (114 in 1961 and 149 in 1962).

Data from field collections during both years readily show that sexually mature fish have a higher degree of infection and harbor a greater cestode burden than do sexually immature ones. During April, May, and June, 1962, 37 (76 percent) of 49 adult fish were infected, but only 41 (50 percent) of 82 immature carp were parasitized. The maximum number of cestodes present in a single adult carp was 1523, while the greatest number recovered from one immature fish was 109. The cause of this significant difference in percentage infection and in cestode burden is unknown. However, it may be associated with the much greater volume of food ingested by the adult fish, thereby enhancing the possibility of their acquiring infected tubificids.

There appears to be little correlation between size of the fish and its ability to harbor *A. iowensis*. During those months when infections occurred, carp ranging in size from 5 to 25 inches were examined and found to be parasitized. Since infections of *Archigetes* do not occur until early spring, the youngest carp in the Iowa River are 5 inches or more in length at this time.

Infections of *A. iowensis* are not related to the sex of the fish. During 1962, 24 (80 percent) of 30 sexually mature male and 20 (77 percent) of 26 adult female carp harbored these parasites.

During the 1962 collections, gravid cestodes were first found in carp on April 21. However, they were not abundant until early May, after which time gravid worms were present until parasites disappeared from fish in July. For example, 12 (12 percent) of the 101 *A. iowensis* collected from 15 infected carp on April 21, were gravid, but none possessed more than a dozen eggs *in utero*. One carp examined on May 6, harbored 1523 parasites; 828 (54 percent) were gravid. Of the 271 cestodes taken from a single carp on May 21, 225 (83 percent) were ovigerous. On June 21, a single carp, harboring 232 parasites, possessed 102 (44 percent) gravid worms.

Details of Iowa River carp collections and the degree of parasitism with *A. iowensis* during 1961 and 1962 are presented in Table 5.

Table 5. Infection of Iowa River carp with _A. iowensis_ during 1961 and 1962

	Number of carp examined	Number infected	Average number of worms per infected fish
1961			
May	18	11 (61%)	114 (1-787)
June	5	4 (80%)	4 (1-11)
July	8	0	0
August	--	--	--
September	12	0	0
October	10	0	0
November	5	0	0
1962			
April	61	34 (56%)	8 (1-63)
May	56	33 (59%)	149 (1-1523)
June	13	10 (77%)	120 (6-301)
July	24	0	0
August	15	0	0
September	19	0	0
October	9	3 (33%)	37 (1-109)
November	6	3 (50%)	11 (2-28)
Totals	261	98 (39%)	

Experimental Studies

Following the discovery of A. iowensis in tubificids (August, 1960), naturally infected oligochaetes harboring mature cestodes of this species were fed to 20 small specimens of five fish species. Each of these fishes (six Catostomus commersoni, five Carassius auratus L., four Cyprinus carpio, three Pimephales promelas Raf., and two P. notatus Raf.) was fed one to 14 infected tubificids. Although fish were examined as soon as 24 hours post-feeding, no live cestodes were recovered.

Despite these negative results, it became apparent that Archigetes did parasitize fish, since in May, 1961, massive infections were encountered in carp. At this time, immature carp were only lightly infected. Consequently, additional feeding experiments were undertaken with larger carp. During the interval of May through August, 1961, and in the months of May and June, 1962, each of 27 adult carp was force-fed nine to 14 mature specimens of A. iowensis (within tubificid hosts). Live cestodes were recovered from only two of these fish, and none were found later than 24 hours post-feeding. The two successful feeding experiments were conducted in May, at a time when natural infections occurred in carp.

Although most experimental feedings were conducted with larvae of A. iowensis within naturally infected oligochaetes, additional experiments involving experimentally reared procercoids were undertaken in August, 1961. Such procercoids, 119 days post-infection, were derived from eggs of A. iowensis from carp. However, when these larvae were fed to carp, results were also negative. No experimentally reared tapeworms have yet been fed to carp during those months when natural infections occur in fish. The feeding experiments conducted to date with large carp are summarized in Table 6.

Although these experiments have not definitely established the manner in which carp acquire infections, there seems to be little doubt that they do so by ingestion of infected tubificids. Examination of food from carp stomachs and intestines from April through November shows that these fish commonly ingest tubificids during these months. The possibility of a second intermediate host for *Archigetes* seems to be slight, indeed. During other experiments it was found that tubificids were able to transmit infections of a related caryophyllaeid species to white suckers. The failure of the feeding experiments involving *A. iowensis* and carp may well be related to the periodicity of this species. This periodicity is discussed in the following section on host-parasite relationships.

Table 6. Summary of experimental feedings of oligochaetes infected with mature *Archigetes iowensis* to carp. Size of fish varied from 12 to 22 inches in total length. Fish of both sexes were used during each month, except in May, 1961

Month	Number of fish used	Number of worms fed each fish	Time, in hours, between feeding and examination	Results
1961				
May	1	17	24	3 mature tapeworms recovered
June	9[a]	10-14	24	Degenerating adults only
July	3[a]	9	48-72	All fish negative
August	2[b]	5-6	48	Both fish negative
1962				
May	4	4-10	24-60	1 live, mature tapeworm from one fish after 24 hours
June	6	10	24	All fish negative
July	2	7	24	2 degenerating adults from one fish; other fish negative

[a]Some fish given pituitary injections 24-72 hours prior to experiments.

[b]Experimentally reared cestodes used in experiments.

HOST-PARASITE RELATIONSHIPS

The lack of recovery of adult worms in the experimental feedings is, very probably, related to the factors responsible for the marked seasonal periodicity of *A. iowensis* in carp. Little is known concerning the seasonal distribution of caryophyllaeid cestodes. Furthermore, according to Dogiel *et al.* (1961): "There is, as yet, very little information about seasonal fluctuations in the occurrence of fish parasites in natural waters." Although many accounts of caryophyllaeid cestodes have appeared in the literature, most involve descriptions of individual species; life history studies are conspicuously sparse. Scheuring (1929) associated the disappearance of *Caryophyllaeus laticeps* from carp in winter with the cessation of feeding by the fish. Wunder (1939) also studied the periodicity of this caryophyllaeid. Although his study was based upon carp from culture ponds where transfers of fish, lime treatment of ponds, and periodic pond drainage undoubtedly affected the yearly infection cycle, certain findings are notable. Wunder found that infection of carp with *C. laticeps* occurred only during the months of April through August. On a monthly basis, percentage infection of fish was: April, 44 percent; May, 54 percent; June, 26 percent; July, 9 percent; and in August, 1 percent. He found the average number of tapeworms per infected fish was greatest in May (April, 73; May, 133; June, 35; July, 6; and in August, 2). The maximum number of cestodes found in a single fish (497), also occurred in May. Infections of carp with *C. laticeps* show many similarities to corresponding infections of these fish with *A. iowensis*. However, Wunder explained the periodicity of *C. laticeps* primarily upon techniques of pond management. It should be again noted that some workers consider the species of *Caryophyllaeus* found in *Cyprinus carpio* to be *Caryophyllaeus fimbriceps*, not *C. laticeps*.

Bauer's (1961) findings differ somewhat from those of Wunder (1939). Bauer found that fingerling carp become infected with *Caryophyllaeus* in autumn, and that such infections persist throughout the winter.

Studies of American caryophyllaeids have added somewhat to our knowledge of seasonal periodicity. Self and Timmons (1955) reported that caryophyllaeid parasites of *Carpiodes carpio* Raf. were probably most abundant in the spring. Although studies conducted by McCrae (1960) and by Mackiewicz (1960) did not indicate a seasonal periodicity for *Glaridacris catostomi* in *Catostomus commersoni*, corresponding infections of Iowa River white suckers do show a distinct periodicity. For example, during my studies in 1962, gravid adults of this species occurred in fish only during April, May, and June. In these months, *Glaridacris* was present in 69 percent of the white suckers examined. Although 130 suckers were examined in the months of July and August, only one (immature) *G. catostomi* was recovered. However, the periodicity of this caryophyllaeid differs from that of *A. iowensis*, since fish parasitized by immature *Glaridacris* are first recovered in September. By November, suckers may harbor many immature worms. Gravid *G. catostomi* were later recovered from suckers collected in January, 1963.

Gravid adults of certain Iowa River caryophyllaeids occur in fish only in mid-summer, while still other species appear to exhibit two distinct peaks during the year. Results of additional studies currently underway indicate that periodicity is quite common among Iowa River caryophyllaeids. _A. iowensis_ appears to show the most pronounced periodicity of these cestodes.

Three caryophyllaeid species have been reported from carp in the United States, namely _Atractolytocestus huronensis_, _Khawia iowensis_ and _Archigetes iowensis_. Of these caryophyllaeids, only _A. iowensis_ appears to exhibit periodicity. Anthony (1958) described _A. huronensis_ from carp in Michigan but gave no details concerning the life history of this species. However, in his 1952 doctoral thesis, Anthony stated that these parasites were recovered from carp each month during the interval May through November. In their description of _K. iowensis_, Calentine and Ulmer (1961) reported that gravid adults were found in Iowa River carp at irregular intervals from April through November. Additional studies during 1962 also show that this caryophyllaeid apparently does not exhibit periodicity.

Apparently _Archigetes appendiculatus_ (=_B. appendiculatum_) is the only other species of _Archigetes_ reported from fishes. Although the life history of this species has not been elucidated, it should be noted that Janiszewska (1950) reported these cestodes from fishes during the months of April and May. Szidat (1937) did not indicate the time of year that he found _Archigetes_ in fish, but stated that these cestodes probably remain in fish only a short time. Perhaps species of _Archigetes_ (other than _A. iowensis_) exhibit periodic infection of the fish host.

Limnological studies on the Iowa River were not conducted in conjunction with the life history of A. iowensis. Possible relationships between certain physical conditions, such as water temperature, and the periodic infection of carp with Archigetes were considered. However, it seems more likely that this periodicity is a result of biological rather than of physical factors. Attempts were made to associate this periodicity with: (1) variations in the availability of infective procercoids, (2) the spawning period of the carp, (3) possible immune responses of carp, (4) possible changes in resistance of carp to cestode infection, and (5) variations in carp feeding habits.

Variations in the availability of infective procercoids

Annelids infected with larval A. iowensis also exhibit a seasonal abundance (Graph 1). As indicated previously, larvae must apparently mature in tubificids in order to infect fish. Although mature larvae in tubificids are most abundant in the fall, attaining a well-defined peak in October, they are present every month of the year. Furthermore, infected annelids were recovered from all areas of river from which fish collections were made.

Quantitative studies on the oligochaete population in the Iowa River were not conducted. However, field collections readily indicated that in both 1961 and 1962, the tubificid population became abruptly reduced in July and continued so through August. This decline of oligochaetes occurs precisely at the time A. iowensis disappears from carp. However, it should be noted that percentage infection of annelids is approximately the same in July (when fish are not infected) as it is in early spring (when fish harbor massive infections). Since tubificids harboring mature

Archigetes were present every month of the year (even though greatly reduced in abundance during July and August), and since food analyses showed carp to feed upon oligochaetes throughout spring, summer, and fall, it was doubted that this tubificid scarcity was responsible for the absence of adults in carp. Nonetheless, an experiment was conducted to test this belief. Two Iowa River carp were each fed (in July, 1962, after A. iowensis had disappeared from fish) with seven naturally infected oligochaetes harboring mature cestodes. These tubificids were collected in June, 1962, at a time when the parasites should have been almost one year of age. The two fish were examined 20 hours post-feeding. One fish was negative; two Archigetes were recovered from the second fish, but both were in process of being digested.

At the time of maximum abundance (fall of the year), larval caryophyllaeids of genera other than Archigetes are infective to fish. Although procercoids of A. iowensis demonstrate a similar peak in the fall, they apparently are not infective to fish at this time.

Larval A. iowensis are clearly capable of producing long-standing infections of the annelid host. Procercoids were maintained in the laboratory for more than 560 days; in nature, larvae are probably capable of remaining in tubificids for even a longer time, because of the lower temperatures during most of the year. Although studies conducted on caryophyllaeids (other than A. iowensis) from the Iowa River indicate that duration of annelid infection may affect the periodicity of adults in fish, this does not appear to be the case with Archigetes.

Seasonal changes in the abundance of larval A. iowensis apparently are not directly responsible for the periodicity of adults in carp. It also appears that this periodicity is not associated with the intermediate host, but rather, is related in some manner to the fish host.

Spawning period of carp

Szidat (1959) discussed the possible role of hormones on abridged life cycles of caryophyllaeids. During 1961, it appeared likely that infections of Iowa River carp with A. iowensis were associated with the spawning period of the fish, and that perhaps this periodicity was caused by a hormonal relationship. In 1961, the duration of fish infection seemed to correspond closely to the spawning period of the fish. Secondly, sexually mature fish showed a higher rate of infection and harbored a greater cestode burden than did immature ones. However, later findings failed to substantiate this viewpoint. For example, gravid carp were occasionally recovered as late as October, yet such fish never harbored Archigetes. In 1962, the peak of cestode population in carp appeared prior to the spawning of the fish. Also, feedings of mature cestodes to gravid carp in June, 1962, failed to produce infections. Experimental feedings of A. iowensis to carp were also conducted subsequent to pituitary injections (in an attempt to elevate the pituitary and gonadal hormone levels equal to those of spawning fish) in June and July, 1961. Again, no live cestodes were recovered from these fish. The pituitary glands used in these experiments were obtained from large carp and suckers. Freshly removed glands were placed in acetone (three changes) for 24 hours, air-dried, and stored whole. For each injection, a dried gland was pulverized and mixed with 6 ml of distilled water just prior to use. Injections, made into the peritoneal cavity of the fish at the base of the pelvic fin, were given 24 to 72 hours prior to the feeding experiments.

Evidently the pronounced periodicity characteristic of the species is not directly associated with the spawning period of the host, nor does it appear to be associated with variations in host sexual hormone levels.

Possible immune responses of carp

A simple immune reaction does not satisfactorily explain the periodic infection of fish. For example, feedings of *A. iowensis* to carp, which apparently had never harbored these parasites, were not successful in July and August (after natural infections had disappeared). If carp develop immunity after once harboring *Archigetes*, one would expect larger, older carp to show a lower rate of infection than smaller, younger fish. Infections of fish with *A. iowensis*, however, demonstrate precisely the opposite, for larger carp harbor the heaviest infections.

The possibility of an age immunity existing was also considered. Young of the year carp (4 to 9 inches in length) never harbored *A. iowensis* in the calendar year of hatching. In the following spring, however, carp as small as 5 inches in length were examined and found to be parasitized. Infections occur in fish of most sizes, but appear to become established only during a specific time interval.

Possible changes in resistance of carp to cestode infection

Although a simple immune response does not appear to explain the periodicity of *Archigetes* in carp, certain findings indicate that resistance of carp to cestode infection does show evidence of variation during the year. These changes of resistance may be sufficient to permit infections of A. iowensis at certain times of the year, but not at others. Evidence supporting this belief is cited below.

On April 6, 1962, members of another caryophyllaeid species were found in Iowa River carp for the first time, but were recovered only from these fish in a very limited area of the river (Iowa Falls impoundment). As many as 95 percent of the carp examined during individual collections in April harbored such cestodes. Most worms were attached to the intestinal mucosa (Figure 28), and were often located in distinct groupings. However, these helminths were recovered from carp no later than May 12, and no completely mature specimens were ever found in these fish. Sexually mature fish have a higher percentage of parasitism with this cestode (as do carp infected with Archigetes) than do immature ones. Thus, during April and May, 1962, 15 (88 percent) of the 17 adult fish and 47 (60 percent) of the 78 immature carp harbored these parasites.

Although no adults of this species were recovered from carp, study of immature individuals (Figure 22) indicates that they probably belong to the genus Monobothrium Diesing, 1863. It is believed that the definitive host of this species may be either bigmouth buffalo, Ictiobus cyprinellus (Val.), or hog suckers, Hypentelium nigricans (LeSueur). Although both of these fish species are now apparently absent from this area of river (because of rotenone poisoning in 1960), attempts are being made to study the taxonomy and life history of this caryophyllaeid on other regions of the Iowa River. Similar parasites were later recovered from hog suckers at unpoisoned areas, but no caryophyllaeids have yet been found in bigmouth buffalo at the same site.

Since Monobothrium sp. was not found in other fish species examined at the Iowa Falls impoundment in April, its presence in carp is believed to be the result of a lowered resistance on the part of the fish, rather than being caused by a lack of host specificity on the part of the parasite. The periodicity of A. iowensis in carp may be the result of the same factor. Since specimens of Archigetes mature in tubificid hosts, and since they remain in carp for a longer time than do those of Monobothrium sp., they are able to produce eggs during their stay in fish.

The presence of many deformed parasites (of both Monobothrium and Archigetes) in carp gives additional evidence of changes in resistance of fish to cestode infection. The number of abnormal parasites found in carp was rather low in early spring, but appeared to increase by June. As previously indicated, those Archigetes found in carp during October and November, 1962, were also highly deformed.

It is not yet known at what time of year infections of A. iowensis are acquired, since fish collections have not yet been possible during the months of December through March. However, field collections seem to indicate that carp acquire their infections in early spring. At the time of the earliest fish collection made to date (April 6, 1962), fish harbored light infections, and no gravid worms occurred. The average number of cestodes per infected fish rapidly increased until May, when maximum infections occurred.

The presence of Archigetes in carp during the fall of 1962 can be explained in one of two ways. Since carp were feeding heavily upon oligochaetes, and since infected tubificids are at their yearly peak in October and November, it is possible that these cestodes were merely passing through the fish. It is noteworthy that those carp examined which had no food in their intestines at this time, were not parasitized. The recovery of immature caryophyllaeids (regularly parasitizing white suckers) from carp during these two months gives support to the viewpoint that these Archigetes were merely passing through carp at the time of examination. Also, the fish examined during these months in 1961, taken from areas where infected oligochaetes were not so abundant, did not harbor Archigetes.

The alternate explanation for the presence of these cestodes in fish during the fall is that the resistance of carp to cestode infection is again decreasing. Although most of cestodes recovered in late fall were abnormal, some of these worms may persist through the winter. On the basis of present knowledge, I believe that infections of Archigetes are normally acquired in early spring.

Although explanations of periodicity based upon changes in host resistance do not seem consistent with the sudden disappearance of cestodes from fish in July, on the basis of present knowledge, this explanation best fits the findings of this study. This low resistance of carp to cestode infection during the spring may, in part, be related to the physiological conditions of carp as a result of overwintering.

Variations in carp feeding habits

The feeding habits of Iowa River carp were studied in order to determine if they might be directly responsible for the periodicity of these cestodes. It should be first noted that although infected tubificids were especially abundant in certain areas, they were present in all regions of river from which fish were collected. Tubificid remains were recovered from carp every month during the interval April through November. Oligochaetes can be readily identified in the intestinal contents of fish, since large areas of cuticle with associated setae remain undigested.

Carp in the Iowa Falls and Alden impoundments, areas where greatest infections of A. iowensis occurred in both oligochaetes and fish, are almost exclusively bottom feeders. Although exogenous plant materials are eaten in large quantities at certain times of the year, aquatic invertebrates and bottom mud predominate as carp food. Tubificids, chironomids and mayfly nymphs were consistently found in the intestines of carp collected in these areas. Mayfly nymphs composed the greatest bulk of animal material found in carp. In late summer and early fall, the intestines of some fish were found to contain only crayfish. Carp feeding further upstream, over sand and gravel in riffle areas, ingested many stonefly nymphs.

Since carp were found to feed upon tubificids throughout the interval of April through November, the periodicity of cestodes in carp is apparently not a result of changes in host feeding habits. However, the low resistance of carp to cestode infection in the spring of the year may possibly be due to altered physiological conditions. Relationships between physiological conditions of fish and cestode infections have received little attention. Furthermore, there are pronounced differences of opinion regarding the winter activities of carp. Schaeperclaus (1933) states that some workers believe that carp go into hibernation at a temperature of 7.5 degrees Centigrade, while others maintain that carp may feed actively at a temperature of 3 degrees Centigrade. Dubinina (1949), studying hibernation of fishes, concluded that carp remain in their lairs throughout the cold months, and being in a state of stupor, cease to feed. She also found that fishes generally lose their trematode and nematode infections during the winter, and that segmented tapeworms undergo destrobilization.

The winter feeding activities of Iowa River carp have not yet been investigated because of the difficulties in securing fish at this time. Carp in an ecologically similar river (Des Moines River) were found to be feeding actively on December 8, 1962, at which time the Iowa River study area was completely ice-covered. Search of the literature reveals that no studies involving the winter feeding habits of carp in rivers have been conducted in Iowa. However, Moen (1954) found that carp in Iowa lakes do considerable feeding in winter. He reported that 100 percent of the winter carp food was of animal origin, with crustaceans and larval dipterans predominating. The role of crustaceans as carp food in the Iowa River, however, is minor because of their relative scarcity.

During attempts to secure Iowa River carp in January, 1963, six white suckers were collected. The food of these fish in the Iowa River is quite similar to that of carp. Four of the six fish examined in January possessed intestines well-filled with food. All of the six fish were infected with caryophyllaeid cestodes, including *G. catostomi*. Additional collections are planned in an attempt to study the winter feeding habits of carp, as well as their parasite burden. Thus, the effect of winter feeding activities of carp upon low cestode resistance in the spring cannot be yet ascertained.

THE *ARCHIGETES*-*BIACETABULUM* RELATIONSHIP

General Account

The most extensive accounts of North American caryophyllaeids are those of Hunter (1927, 1929, and 1930), who described five genera and nine species. Other reports on American caryophyllaeids are almost exclusively of a descriptive nature. Mackiewicz (1961b), in a summary of the North American caryophyllaeids, recognized 11 genera and 19 species (three genera and four species have since been reported). That many species are as yet undescribed is indicated by recent publications. For example, Mackiewicz (1961a) reported the presence of six new caryophyllaeid species, including three new genera, from the catostomid fish *Catostomus commersoni* in New York. McCrae (1961) also mentioned additional species from the same host fish in Colorado. Furthermore, during the present study, cestodes which may represent as many as eight additional species (distinct from those found by Mackiewicz and McCrae) have been recovered from seven species of cyprinid and catostomid fishes in Iowa. It is evident that additional study is needed before taxonomic relationships of caryophyllaeids, especially the American forms, can be elucidated.

The genus *Archigetes* Leuckart, 1878, is relatively well-known in Europe. Although Ward (1911) and Hunter (1930) mentioned *Archigetes*, no undisputed members of this genus were recorded from North America until the description of *A. iowensis* appeared in the literature. Wisniewski's (1930, page 77) generic diagnosis (translation) of *Archigetes* follows:

> Small (2.5 to 6 mm) caryophyllaeids parasitic in the body cavity of tubificids, especially *Limnodrilus hoffmeisteri*,

> and reaching sexual maturity in the procercoid stage. A more or less distinct bothrium is present on the dorsal and ventral surfaces of the scolex. A cercomer, characteristic of the procercoid stage, is present, bearing 6 embryonic hooks at posterior. Frontal glands not extending posterior to scolex. Uterine glands present or absent. Glandular ootype present. Genital opening covered by cuticle. Vagina possessing a distinct seminal receptacle. Cirrus opening into a genital cloaca. Cirrus pouch round. Well-developed excretory system, in the form of an irregular net, appearing at the posterior as 6 to 7 ampullae. Eggs operculate, do not hatch in free state. Host infected by ingestion of embryonated eggs. Ciliated oncosphere lacking.

In contrast to *Archigetes*, the genus *Biacetabulum* Hunter, 1927, has primarily an American history. Apparently the only species of *Biacetabulum* known from Europe is *B. appendiculatum*, here considered as properly belonging to the genus *Archigetes*. Hunter (1930) separated members of *Biacetabulum* from *Archigetes* because of their differences in scoleces, excretory systems, hosts, and their lack of a cercomer. Hunter's (1927, page 21) generic diagnosis of *Biacetabulum* follows:

> Caryophyllaeinae with well defined scolex, varying but little in shape, bearing one pair of well defined acetabular suckers, with or without additional loculi. Cirrus opens into the utero-vaginal canal before it reaches the superficial atrium (as in *Caryophyllaeides*). Ovary "H" shaped, entirely medullary. Uterine coils extend anteriorly to cirrus sac, with a maximum longitudinal extent of one-fourth of testicular field, usually less. Terminal excretory bladder and external seminal vesicle present. Post-ovarian vitellaria present. Parasitic in Catostomidae. Development unknown.

At the present time most caryophyllaeid genera are differentiated on the basis of: (1) nature of the scolex, (2) number of genital apertures, (3) shape of the ovary, (4) presence or absence of an external seminal vesicle, and (5) extent of the uterus in relation to the cirrus pouch. However, in keys to the genera of caryophyllaeids, namely those of Hunter (1930), Fischthal (1951), Wardle and McLeod (1952), and

Yamaguti (1959), Archigetes is distinguished from other genera on the basis of its possessing a cercomer or by the type of host in which it is found, but not by those morphological characteristics listed above. With the discovery of A. appendiculatus in fishes, certain workers, namely Szidat (1937), Janiszewska (1950), Yamaguti (1959), and Kulakowskaja (1961), listed this cestode as a species of Biacetabulum. Because of the differences of opinion regarding the taxonomic status of Archigetes and Biacetabulum, these genera must be re-evaluated with respect to morphology and life history.

Life History of Biacetabulum macrocephalum

Concurrent with the study on A. iowensis, the life history of Biacetabulum macrocephalum was also investigated. McCrae (1962) described this species from Catostomus commersoni in Colorado; I found it in the same host fish in the Iowa River. Only a summary of this study, as it relates to the taxonomic problems discussed above, will be included here.

Gravid adults (Figures 20 and 21) occur in Iowa River fish only during the months of June, July, and August. Infection of suckers attains a maximum in July, at which time 25 percent of these fish harbor Biacetabulum. Immature forms occur in fish during the fall, winter, and spring.

Eggs shed by gravid worms show no distinct differences from those of A. iowensis either in their size or shape, time of embryonation, or nature of the oncosphere. Freshly shed eggs average 35 by 50 microns, time of embryonation is 14 days, and the oncospheres average 20 by 39 microns.

L. hoffmeisteri and T. tempeltoni were exposed to embryonated eggs of B. macrocephalum. Some experiments (conducted in similar fashion to those described for A. iowensis) involved the exposure of both tubificid species in a common container, while in others, each species was exposed separately. Infections developed only in T. tempeltoni. During 12 experiments involving T. tempeltoni, these tubificids became infected in all of them, with a maximum infection of 95 percent in a single experiment. In nature, larvae of Biacetabulum were likewise recovered only from T. tempeltoni.

Oncospheres hatch in the intestine of the tubificid and usually penetrate the gut wall between segments 21 to 34. Embryos move actively within the coelom of the oligochaete, and by six days have migrated to the gonadal region. Most larvae localize between segments 11 to 18, some being found within the posterior seminal vesicle, while others occur within the coelom of the host. Development now proceeds rapidly and by 14 days post-infection, procercoids average 0.32 mm in length. At this time, the ovoid larvae show early stages of cercomer formation (Figure 16). Dorsal and ventral bothria become evident between 18 and 26 days (Figure 17); by the latter age a nuclear mass is present posteriorly. Procercoids now average 0.74 mm in length, and by 62 days, they are 1.14 mm long. The excretory system develops between 35 and 40 days. Rudiments of gonadal organs are formed by 62 days (Figure 19), but no differentiation as to specific structures can be observed. Such larvae are apparently fully developed, since those over 100 days post-infection show no further differentiation nor any significant increase in size.

In October, 1962, two white suckers, collected in nature from a stream where previous examination of fish showed that Biacetabulum was absent, were fed with experimentally reared procercoids (within tubificids) 62 days of age. Apparently normal worms, clearly recognizable as Biacetabulum, were recovered from both fish 20 hours post-feeding.

Morphological Comparisons

On the basis of these preliminary studies, those differences between Archigetes and Biacetabulum, which some workers have considered as significant (hosts and the presence or absence of a cercomer) are here considered to be differences between procercoid and adult stages and hence, not of generic significance. Experimental studies with these and five other caryophyllaeid species clearly demonstrate that procercoids (characterized by the possession of a cercomer in all cases) are found in oligochaete annelids. There are, however, certain differences between these genera.

Excretory system

Members of the genus Archigetes were long thought to possess a unique excretory system. Since an excretory bladder is present in those caryophyllaeids from fish hosts, and since members of the genus Archigetes (from tubificids) lack this structure, some workers consider this difference to be significant. Examination of procercoids of A. iowensis and B. macrocephalum (as well as those of several other caryophyllaeid species) shows that they do not possess excretory bladders. In caryophyllaeid procercoids, the excretory ducts coalesce at the posterior of the body and empty into the cavity surrounding the proximal end of the cercomer. In fish hosts, following the loss of the cercomer, this cuticular-lined cavity forms the bladder.

In both procercoid and adult stages of *A. iowensis* and *B. macrocephalum*, the excretory ducts in the scolex consist of a very close-meshed network in the cortical parenchyma. One large medullary duct extends longitudinally on each side of the median scolex depressions, and in the neck region, anastomoses with the cortical ducts. A network of small, cortical ducts, originating at the posterior of the body, extends anteriorly. In the neck region, the ducts from the scolex anastomose with these ascending ducts. The large ducts, thus formed, descend to the posterior of the body, and are located in immediate proximity to those of the ascending network. Thus, the excretory ducts in the body proper appear in closely associated pairs, one member of each pair (the descending duct) being considerably larger than the other.

There is, however, one major difference in the organization of excretory ducts in *Archigetes* and *Biacetabulum*. In *Archigetes* no longitudinal orientation of the excretory ducts is present. The excretory system of *A. iowensis* is essentially similar to that of *A. sieboldi* (illustrated by Wisniewski in 1930) and to that of *A. appendiculatus* (as shown by Mrázek in 1897). In *Biacetabulum*, on the other hand, the cortical excretory ducts are distinctly oriented longitudinally. Study of living larvae and sections of adult *B. macrocephalum* shows that ten pairs of ducts normally occur. Other species of *Biacetabulum* exhibit a similar orientation of excretory ducts; this difference appears to be constant at the generic level.

Scolex

Although the nature of the scolex is commonly used in distinguishing genera of caryophyllaeid cestodes, members of certain genera (Glaridacris, for example) exhibit considerable variation in this respect. The terminology used to describe scolex depressions in some caryophyllaeid genera also varies greatly. Wisniewski (1930, page 77) referred to the scolex of Archigetes as "bothriate." Hunter (1927, page 21) described the median scolex depressions in Biacetabulum as "acetabula." Hyman (1951, page 377) referred to the scolex of Biacetabulum as possessing "two deep rounded suckers resembling acetabula accompanied or not by four shallow bothria." Wardle and McLeod (1952, pages 543, 544, and 548) used the term "pseudobothriate" with reference to the scoleces of Archigetes, Glaridacris, and Biacetabulum. Yamaguti (1959, page 21) designated the scolex depressions of Biacetabulum as "saucer-like."

Hyman (1951) considered the distinguishing features of bothria to be their shallow shape, weak muscularity, and the absence of an inner bounding muscular layer. She also characterized acetabula as hemispherical depressions with radial muscle fibers. Wardle and McLeod (1952) indicated that in acetabula, the muscular wall is separated from the underlying tissues by a basement membrane.

In this paper, scolex depressions which are not hemispherical and are not bounded by a distinct layer of radial muscle fibers, but which are, however, uniform in depth (as the median depressions in A. iowensis) are considered as bothria. Those depressions which are not uniform in depth (as the lateral depressions in Archigetes and Biacetabulum) are termed loculi. Hemispherical depressions with distinct radial muscle fibers, separated from the underlying tissues by a basement membrane, are called acetabula. The median scolex depressions in *Biacetabulum* are considered to be acetabula.

Meyer (1960), in his study of pseudophyllidean cestodes of the genus *Marsipometra* Cooper, 1917, found that the shape of the scolex and the nature of the bothria are in a large measure dependent upon the manner of handling of the specimens, or upon fixation procedures. Such variations in the shape of the scolex due to handling are also exhibited by caryophyllaeid cestodes. In freshly fixed specimens of *A. iowensis* and *B. macrocephalum*, the shape of the scolex for members of each species assumes a consistent, characteristic shape (Figures 24 and 26). However, if cestodes are allowed to remain in distilled water, tap water, or saline for a time prior to fixation, the nature of the scolex and shape of the depressions (Figures 25 and 27) are considerably different from those of freshly fixed specimens. The median depressions become enlarged and very shallow, while the lateral loculi are now indistinct or completely absent. In general, the median scolex depressions in *Biacetabulum* are more muscular and better defined than those in *Archigetes*.

Reproductive system

Members of *Archigetes* and *Biacetabulum* are similar in that both possess a single genital aperture, into which the cirrus and utero-vaginal canal open.

These genera differ with respect to the shape of the ovary and the nature of the external seminal vesicle. In *Archigetes* the ovary is generally dumbbell-shaped, while in *Biacetabulum*, it is H-shaped. An external seminal vesicle is characteristic of both genera. However, in *Biacetabulum* this structure is elongate, possessing relatively thin walls and appears primarily as an enlargement of the vas deferens. The thick-walled vesicle in *Archigetes* is pyriform and appears much more distinct than in the former genus. In *Archigetes* the vesicle is either attached to the cirrus pouch or is in close proximity to it, while in *Biacetabulum* the seminal vesicle is not closely associated with the cirrus pouch.

In members of *Biacetabulum*, the uterus extends far anterior to the cirrus pouch, while in *Archigetes* the uterine coils may or may not extend anterior to the cirrus pouch, depending upon the species. Also, in some species of *Archigetes* (from tubificids) the extent of the uterus depends upon the number of eggs that it contains.

In *Biacetabulum*, the follicles of the preovarian vitellaria are not contiguous, lateral to the ovarian wings, with the postovarian follicles. In *Archigetes*, these follicles may or may not be continuous.

No significant differences in the testes between these two genera can be distinguished, except that cestodes of *Biacetabulum* (generally of a larger size than those of *Archigetes*) have a greater number of testes.

Life History Comparisons

In addition to the morphological differences indicated above, *A. iowensis* and *B. macrocephalum* show four pronounced differences in their life histories, namely differences in (1) intermediate hosts, (2) definitive hosts, (3) larval development, and (4) adult periodicity.

Intermediate hosts

Although detailed experiments have not yet been conducted concerning specificity for intermediate hosts, it has been established that *A. iowensis* parasitizes *L. hoffmeisteri* but not *T. tempeltoni*, while *B. macrocephalum* infects *T. tempeltoni* but not *L. hoffmeisteri*. Since intermediate hosts for other American species of *Biacetabulum* are unknown, it is not possible to speculate upon this specificity at the generic level. Although *A. iowensis* appears to exhibit strict specificity for its tubificid host, certain European species of *Archigetes* (*A. sieboldi* and *A. appendiculatus*) are reported from oligochaetes of the genus *Tubifex* as well as those of *Limnodrilus*.

Additional information relating to intermediate host specificity was presented by McCrae (1961), in a brief account of his experimental studies involving three caryophyllaeid species (*Glaridacris catostomi*, *G. oligorchis*, and *Hunterella nodulosa* Mackiewicz and McCrae, 1962). He exposed embryonated eggs of these caryophyllaeids to three tubificid species, namely *L. hoffmeisteri*, *L. udekemianus*, and *Tubifex tubifex*. McCrae found that infections with these cestodes occurred only in *L. udekemianus*. My experimental results, insofar as *G. catostomi* and *H. nodulosa* are concerned, do not corroborate McCrae's findings. I exposed embryonated eggs of these caryophyllaeids to *L. hoffmeisteri* and *T. tempeltoni*. *G. catostomi* developed in both tubificids, while *H. nodulosa* developed only in *L. hoffmeisteri*. One larva, morphologically similar to experimentally reared *G. catostomi*, was found in a naturally infected

L. udekemianus from the Iowa River. Additional studies, still in progress, tend to show that while certain caryophyllaeids exhibit a strict specificity for their tubificid intermediate hosts, others are less specific, being capable of development in oligochaetes of both *Tubifex* and *Limnodrilus*.

Definitive hosts

Those species of *Archigetes* reported from fish hosts (*A. iowensis* and *A. appendiculatus*) parasitize cyprinid fishes. *A. iowensis* is found in *Cyprinus carpio*; *A. appendiculatus* (=*B. appendiculatum*) is reported from *Tinca tinca*, *Abramis brama*, *A. sapa*, *Barbus barbus*, *Aspius aspius*, and from *Cyprinus carpio* (see Szidat, 1937; Janiszewska, 1950; and Kulakowskaja, 1961).

Four described species of *Biacetabulum* are known from America; all parasitize catostomid fishes. Hunter (1927 and 1929) described *B. infrequens* from *Moxostoma anisurum*, *B. meridianum* from *Erimyzon sucetta*, and *B. giganteum* from *Ictiobus bubalus* and from *Ictiobus* sp. Mackiewicz (1961a) reported an additional species of *Biacetabulum* (as yet undescribed) from the catostomid fish *Catostomus commersoni* in New York. McCrae (1961) also reported two species of this genus from the same host fish in Colorado. He later (1962) described one of these, *B. macrocephalum*. Self and Timmons (1955) reported Hunter's *B. meridianum* from *Carpiodes carpio* in Oklahoma. In the Iowa River, species of *Biacetabulum* are also limited to catostomid fishes (*M. anisurum*, *C. commersoni*, and *Carpiodes* spp.).

A species of Biacetabulum has been reported from a siluroid fish; Johnston and Muirhead (1950) described B. tandani from Tandanus tandanus in Australia. Since the description of this species was made without the use of sectioned material, the relationship between testes, vitellaria, and parenchymal muscles is unknown. For this reason, my references to Biacetabulum are limited to the more adequately described American species.

On the basis of present knowledge, it appears that members of Archigetes are limited to cyprinid fishes, while members of Biacetabulum (with the possible exception of B. tandani) are restricted to catostomid fishes.

Larval development

A. iowensis and B. macrocephalum show distinct differences in their degree of development in their intermediate hosts. Procercoids of Biacetabulum do not attain sexual maturity in tubificids. Larvae of all species of Archigetes, on the other hand, consistently mature and are capable of becoming ovigerous in these hosts. Such progenetic development is associated with only one other caryophyllaeid genus, having been reported by Yamaguti (1934) for one member of the genus Glaridacris, namely G. limnodrili. However, included in his description of this species is the following (page 11): "The distal part of the uterus . . . opens with the vagina into the genital atrium just behind the male aperture." It should be noted that one of the distinguishing features of Glaridacris is the lack of such a common genital atrium; male and female systems open separately on the ventral surface of the worm. However, a superficial depression may be present, as in G. catostomi. Yamaguti's illustration appears to show a single genital aperture. This cestode resembles Archigetes in other ways, namely (1) nature of its scolex, (2) shape of its ovary, (3) nature of its seminal vesicle, and (4) presence of vitellaria lateral to the ovarian wings. Also, the well-known American species of Glaridacris have never been demonstrably shown to parasitize fishes other than those of the family Catostomidae. Yamaguti found G. limnodrili in the cyprinid fish Pseudogobio esocinus (which he lists as the "type" host) and in a cottid fish, Misgurnis fossilis. Possibly Yamaguti was in error in his placement of this species in the genus Glaridacris. Examination of his preparations has not been possible at this time, but study of them in the future may aid in establishing the taxonomic position of this species. Thus, whether or not all caryophyllaeids exhibiting progenetic development can be ascribed to a single genus, Archigetes, is not yet known.

Adult periodicity

Although both A. iowensis and B. macrocephalum show a seasonal periodicity, each species has a characteristic cycle. Gravid adults of A. iowensis occur in fish during April, May, and June, but apparently are not infective to fish during summer and fall. Gravid adults of B. macrocephalum are present in fish only in the months of June, July, and August. Immature Biacetabulum are found in fish during the fall, winter, and spring.

On the basis of these morphological and life history studies, I believe that both Archigetes and Biacetabulum represent separate and distinct genera.

DIAGNOSES

The taxonomic position of caryophyllaeid cestodes is subject to varying interpretations. Since the cestode affinities of these worms have been clearly demonstrated, they cannot be accepted as members of the Cestodaria. Two interpretations currently prevail; caryophyllaeids are considered as a family of the order Pseudophyllidea or as an independent order of the Cestoda. I am inclined to the latter viewpoint. Since caryophyllaeids do not possess a ciliated embryo (typical of the pseudophyllidean tapeworms), and since intermediate hosts for caryophyllaeids invariably appear to be oligochaete annelids (whereas arthropods usually serve as such hosts in the Pseudophyllidea), it would seem that these cestodes possess a distinct and characteristic type of life cycle, as McCrae (1961) emphasized.

Three ordinal diagnoses of Caryophyllidea have appeared in the literature, namely those of Mola (1929), Wardle and McLeod (1952), and Yamaguti (1959). Although not sufficiently explicit concerning the reproductive openings of these helminths, that proposed by Wardle and McLeod is preferred. Their diagnosis is emended (changes or additions indicated by underscoring) as follows:

> Of the class Cestoda. Small forms possessing only one set of reproductive organs. Holdfast undifferentiated, or with grooves sometimes sufficiently broad to simulate bothria. One or two genital apertures on the midventral body surface; cirrus and utero-vaginal canal may open separately or into a common aperture, depending upon the genus. Yolk glands cortical or medullary, or partly cortical and partly medullary, according to subfamily. Adults parasitic in certain families of fresh-water fishes, but certain species may become ovigerous in fresh-water

> annelids. Eggs operculate; oncosphere without cilia. Procercoids developing in oligochaete annelids.

Although many taxonomic problems of the Archigetes-Biacetabulum complex remain unanswered, results of this study indicate that both genera should be retained as separate and distinct taxons. The generic diagnosis (translation) of Archigetes formulated by Wisniewski (1930) is emended (changes or additions indicated by underscoring) as follows:

> Small (1 to 6 mm) caryophyllaeids attaining sexual maturity in the body cavity, seminal vesicles, or ovisac of tubificids (especially Limnodrilus hoffmeisteri), or within the intestinal tract of cyprinid fishes. A more or less distinct bothrium is present on each the dorsal and ventral surface of the scolex. Cestodes from oligochaetes with cercomer, bearing six embryonic hooks. Frontal glands not extending posterior to scolex. Uterine glands present or absent. Glandular ootype present. Genital aperture normally covered by cuticle in cestodes from oligochaetes. Vagina possessing a distinct seminal receptacle. Cirrus pouch round. Cirrus, together with utero-vaginal canal, opening into common genital atrium on ventral surface. Pyriform external seminal vesicle large, with thick walls. Ovary dumbbell-shaped. Preovarian vitellaria primarily in lateral bands and may be contiguous, lateral to ovarian wings, with the postovarian follicles. Excretory system in the form of an irregular net. Cestodes from fish possessing a cuticular-lined excretory bladder. Eggs operculate; oncosphere without cilia. Procercoids developing in oligochaete annelids.

A study of the species assigned to the genus Archigetes has been undertaken. Twenty-three whole mount preparations were obtained from Polish collections through the courtesy of Dr. J. S. Mackiewicz, Albany, New York, and from Prof. Dr. W. Stefanski, Warsaw, Poland. Of these, seven are labeled A. appendiculatus, six A. cryptobothrius, one A. sieboldi, five B. appendiculatum, and four are labeled simply, Archigetes. Seven slides from Dr. Mrázek's collection were obtained through the kindness of Prof. Dr. O. Jírovec, Prague, Czechoslovakia. However no specific designations are given for these specimens. Correspondence with Dr. E. Marcus in Brazil indicates that the specimens of A. sieboldi from his 1942 study are no longer extant.

Four species of Archigetes (A. appendiculatus, A. cryptobothrius, A. brachyurus, and A. iowensis) are readily distinguishable. The single specimen labeled A. sieboldi cannot be differentiated from those labeled A. appendiculatus. Study of literature, namely that of Wisniewski (1930), Mrázek (1897), Marcus (1942), and Janiszewska (1950), indicates that both are probably distinct species. Until studies prove otherwise, A. sieboldi is retained as a distinct species. A key to the species of Archigetes has already appeared in print (Calentine, 1962).

SUMMARY AND CONCLUSIONS

1. Life history stages of the caryophyllaeid cestode, *Archigetes iowensis* Calentine, 1962, have been studied experimentally and in naturally infected hosts during 1961 and 1962. Although representatives of ten species of cyprinid and of ten species of catostomid fishes were examined, gravid *A. iowensis* were limited to carp (*Cyprinus carpio* L.). Carp from five rivers and from six lakes in Iowa were examined; these cestodes were present only in fish from the Iowa River.
2. Carp infections demonstrate a distinct periodicity. These fish were examined during the months of April through November; normal worms were recovered only during April, May, and June. During the month of June, infection of fish may be as high as 80 percent. Sexually mature carp have a higher percentage infection, and harbor a greater cestode burden than do immature fish. The maximum number of parasites recovered from a single fish was 1523. Infected carp ranged in size from 5 to 25 inches in total length.
3. Cestodes may either be attached to the gut mucosa of the fish, or may occur free in the intestinal contents. Damage to the fish host is apparently limited to local destruction of gut epithelium.
4. Natural infections of larval *A. iowensis* were found only in the tubificid oligochaete, *Limnodrilus hoffmeisteri* Claparède. Such infections occur throughout the year, attaining a well-defined peak in October, at which time approximately 5.4 percent of these annelids are infected. Procercoids may become progenetically developed within

tubificids, but, in nature, such worms never comprise more than 3.2 percent of the population.

5. Eggs shed by cestodes from carp are undeveloped, and require 14 days in water to undergo embryonation. Eggs of progenetic procercoids (within tubificids) may be deposited within the seminal vesicles of this host, may be retained *in utero*, may accumulate within the body parenchyma, or may be deposited between layers of cuticle in the region of the genital pore. Development of oncospheres is similar whether eggs are derived from cestodes of carp or are taken from cestodes of tubificids. However, eggs within the seminal vesicles of oligochaetes do not undergo embryonation; those retained within the body of the parasite or within a cuticular sac are significantly larger and may contain well-developed, viable oncospheres.

6. Oncospheres developed from eggs of cestodes from fish may remain viable for a maximum of 80 days in water. Further development does not occur until embryonated eggs are ingested by a suitable tubificid intermediate host.

7. *Tubifex tempeltoni* Southern, 1909, *Dero limosa* Leidy, 1880, and *L. hoffmeisteri* were exposed to embryonated eggs of *A. iowensis* (from fish hosts); infections occurred only in *L. hoffmeisteri*. During four feeding experiments, 31 to 62 percent of these annelids acquired infections. Specimens of *L. hoffmeisteri* also became infected when exposed to embryonated eggs of *A. iowensis* from tubificids.

8. Development of larvae is similar whether eggs are derived from

cestodes of carp or are taken from parasites of tubificids. Hatching of the oncosphere and its penetration of the oligochaete's intestinal wall were not observed, but embryos were seen within the body cavity less than one hour after exposure to viable eggs. Evidence indicates that penetration occurs between segments 15 to 28. Larvae (procercoids) then migrate anteriad to the seminal vesicles, where development normally occurs.

9. A cercomer, bearing the embryonic hooks, is first evident between 25 and 32 days. Bothria and outline of the scolex, apparent after 40 days, appear almost completely formed by 50 days. Primordia of testes are present after 40 days, and by 60 to 70 days, all gonads are fully developed. The excretory system is formed between 40 and 50 days. Growth of procercoids is greatest from 15 to 50 days, post-infection.

10. Egg production by procercoids is very limited and appears to vary in accordance with the source of eggs. Those procercoids developed from eggs of cestodes from carp do not become gravid even after 560 days in experimental infections. One procercoid derived from eggs of cestodes from tubificids became ovigerous by 100 days. In nature, gravid procercoids appear limited to the interval of March through August, but are never abundant. Factors initiating egg production by these larvae are not understood.

11. Since progenetic development of procercoids within tubificids is not of common occurrence, the relatively high percentage infection of fish and oligochaetes in nature indicates that the fish host plays a

very important role in the life cycle of this species.

12. Procercoids of *A. iowensis* are responsible for several pathological effects on the tubificid host. Infected annelids rarely produce ova; spermatozoa may be completely lacking in the posterior seminal vesicle due to the presence of cestodes.

13. Experimental feedings of naturally and experimentally infected oligochaetes to carp did not give conclusive evidence concerning the manner in which fish acquire infections of *A. iowensis*. However, there appears to be little doubt that carp become infected by ingesting parasitized tubificids. The failure of most experimental feedings of cestodes to fish may be related to the periodicity of the adults.

14. Attempts were made to associate the periodicity of adults in fish with: (1) variations in the availability of infective procercoids, (2) possible immune responses on the part of carp, (3) possible variations in resistance of carp to cestode infections, and (4) variations in carp feeding habits. Evidence indicates that variation in resistance of carp to cestode infection may be responsible for this periodicity.

15. An experimental life history study of *Biacetabulum macrocephalum* McCrae, 1962, is summarized. Gravid adults occur seasonally in Iowa River white suckers (*Catostomus commersoni* Lacépède). Embryonated eggs of this caryophyllaeid were exposed to the tubificids *L. hoffmeisteri* and *T. tempeltoni*; infections occurred only in *T. tempeltoni*. Larvae (procercoids) develop either within the seminal

vesicles or within the coelom of the oligochaete host, and apparently are fully developed after 62 days. Procercoids possess a scolex typical of the adult worm, but only primordia of gonads occur. Procercoids 62 days of age are infective to fish.

16. Members of the genera *Archigetes* and *Biacetabulum* are compared and contrasted morphologically; differences are noted in the nature of their scoleces, ovaries, seminal vesicles, and excretory systems.

17. *Archigetes* and *Biacetabulum* are retained as separate and distinct genera. The diagnosis of *Archigetes* is emended; five species (*A. sieboldi*, *A. appendiculatus*, *A. brachyurus*, *A. cryptobothrius*, and *A. iowensis*) are recognized.

18. The ordinal diagnosis of Caryophyllidea is emended.

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PLATES

Abbreviations, used in the illustrations to follow, are explained below:

ASV-anterior seminal vesicle
BV-blood vessel
C-cirrus
CE-cercomer
CH-chloragen tissue
CO-coelom
CP-cirrus pouch
DV-dorsal vessel
ED-ejaculatory duct
EH-embryonic hooks
I-intestine
O-ovary
OV-ovisac
P-penis
PA-parasite
PSV-posterior seminal vesicle
PR-prostate
ST-spermatheca
SV-seminal vesicle
T-testis
U-uterus
UVC-utero-vaginal canal
VD-vas deferens
VG-vitelline gland
VV-ventral vessel
V-vagina

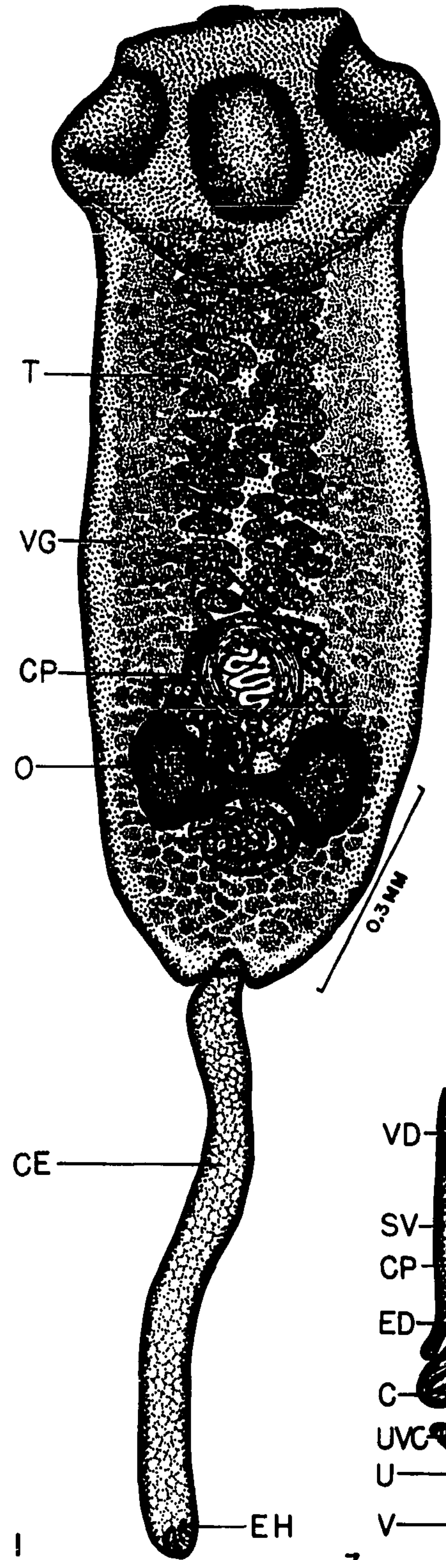
Plate I

Figures 1 through 3. Gravid _Archigetes_ _iowensis_. (Natural infections)

Figure 1. Procercoid from _Limnodrilus_ _hoffmeisteri_

Figure 2. Adult from _Cyprinus_ _carpio_

Figure 3. Sagittal section through genital opening of adult from carp



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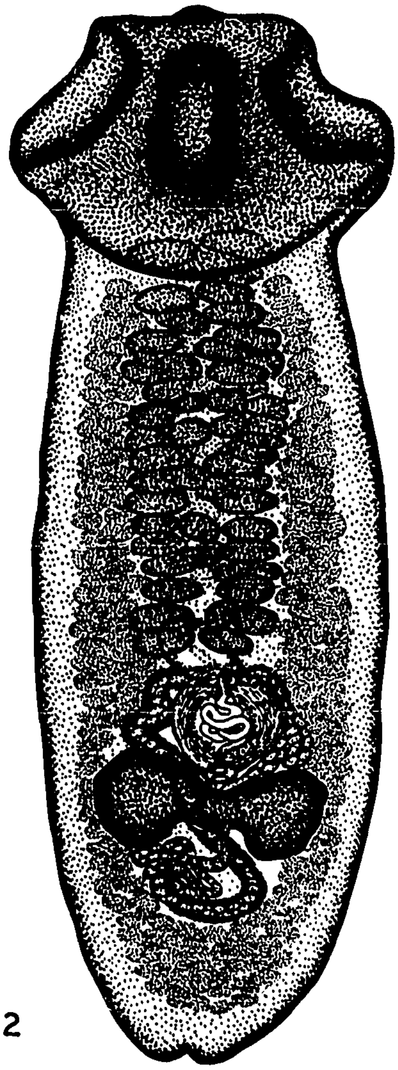

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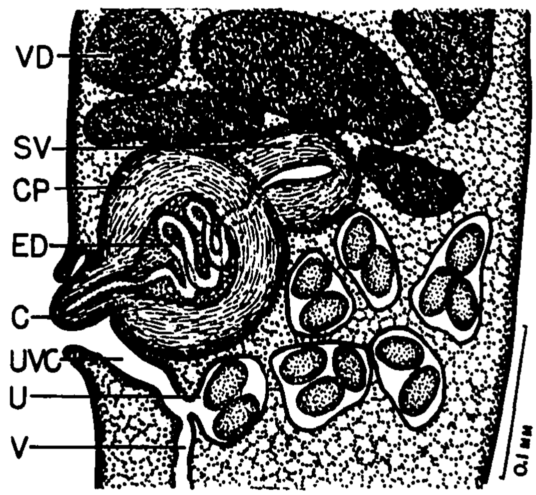

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Plate II

Figures 4 through 7. Oncosphere development in _Archigetes iowensis_. (Eggs shed by cestodes from carp; scale as in Figure 4)

Figure 4. Freshly shed egg

Figure 5. Egg after 5 days in water

Figure 6. Egg after 10 days in water

Figure 7. Egg after 14 days in water. (Oncosphere fully developed)

Figures 8 through 12. _Archigetes iowensis_, procercoids experimentally reared in _Limnodrilus hoffmeisteri_. (Ages given in days, post-infection; scale as in Figure 12)

Figure 8. Procercoid at 32 days

Figure 9. Procercoid at 40 days. (Minimal development in single infections)

Figure 10. Procercoid at 40 days. (Maximal development in single infections)

Figure 11. Procercoid at 50 days

Figure 12. Procercoid at 60 days. (Development nearly complete)

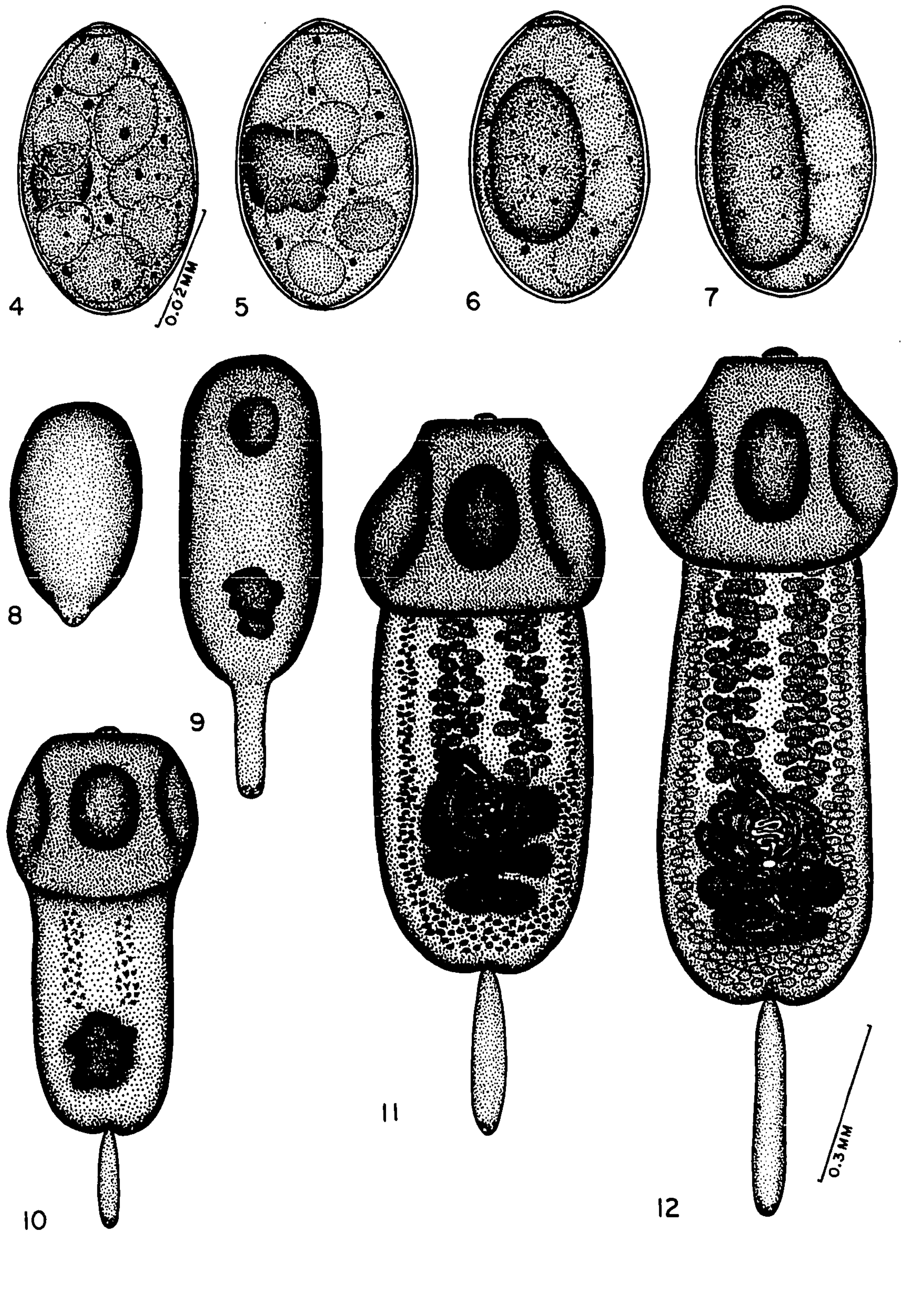
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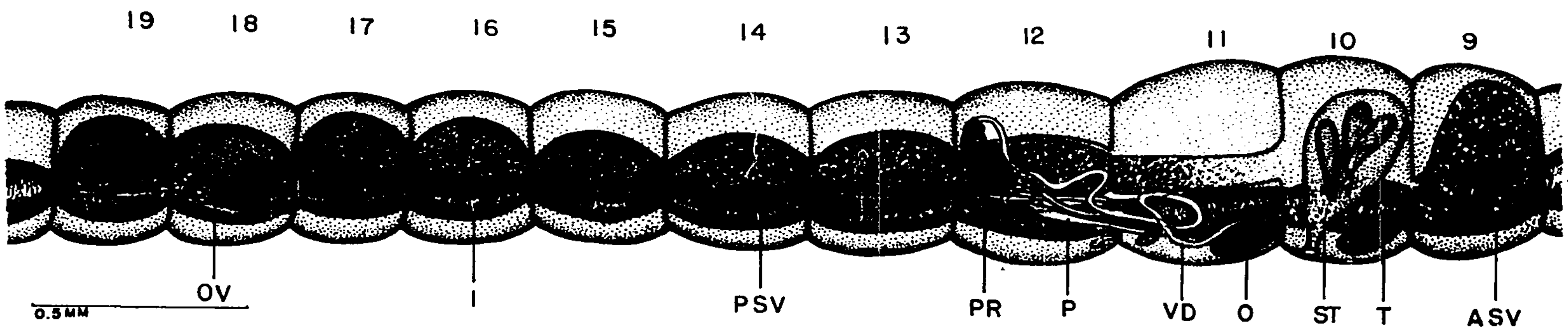
Plate III

Figure 13. Uninfected *Limnodrilus hoffmeisteri*, lateral view. (Paired reproductive structures [testes, ovaries and their respective ducts] are shown only on the right side of the body)

Figure 14. Cross section of uninfected *L. hoffmeisteri*, at level of segment 15

Figure 15. Cross section of infected *L. hoffmeisteri*, at level of segment 15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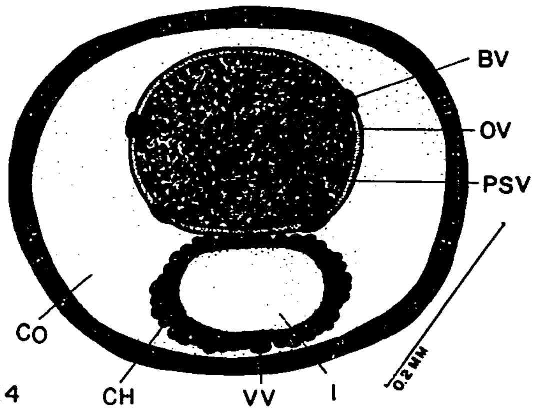
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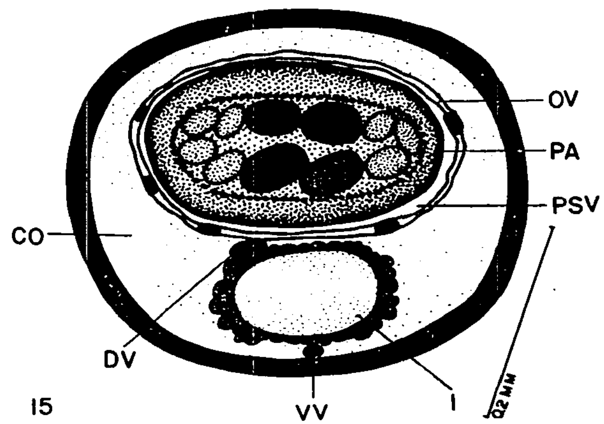
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Plate IV

Figures 16 through 19. Procercoids of <u>Biacetabulum macrocephalum</u> experimentally reared in <u>Tubifex tempeltoni</u>. (Ages given in days, post-infection; scale as in Figure 19)

Figure 16. Procercoid at 14 days

Figure 17. Procercoid at 22 days

Figure 18. Procercoid at 36 days

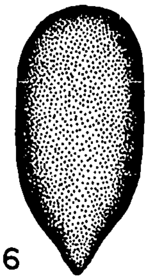
Figure 19. Procercoid at 62 days. (Development complete)

Figures 20 and 21. <u>Biacetabulum macrocephalum</u>, adult. (Scale as in Figure 20)

Figure 20. Scolex

Figure 21. Reproductive complex

Figure 22. <u>Monobothrium</u> sp. (Immature specimen from carp)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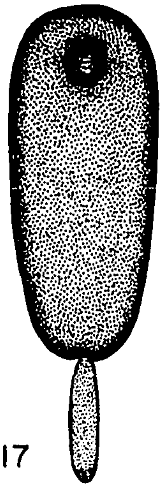
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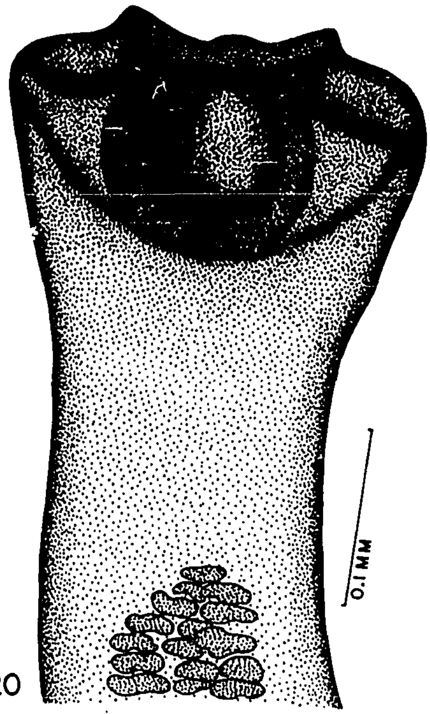
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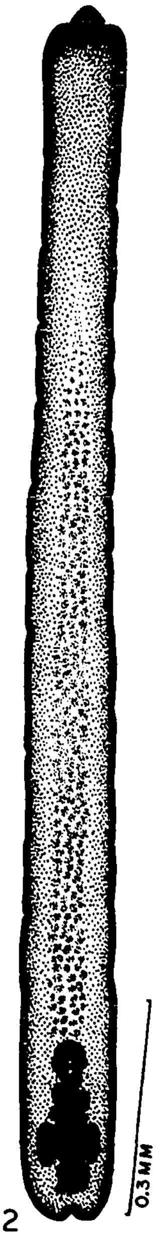
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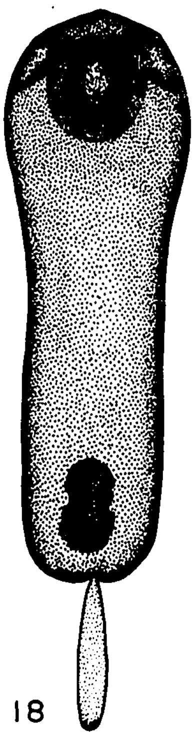
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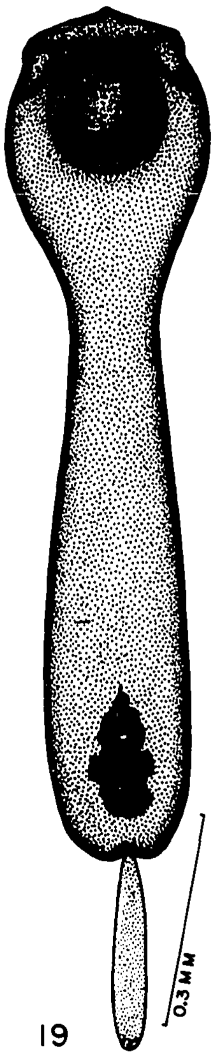
19


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Plate V

Figure 23. Gravid _Archigetes iowensis_ from _Linodrilus hoffmeisteri_. (Note retention of eggs and accompanying degeneration of organs)

Figures 24 and 25. _Biacetabulum macrocephalum_, adult. (Scale as in Figure 25)

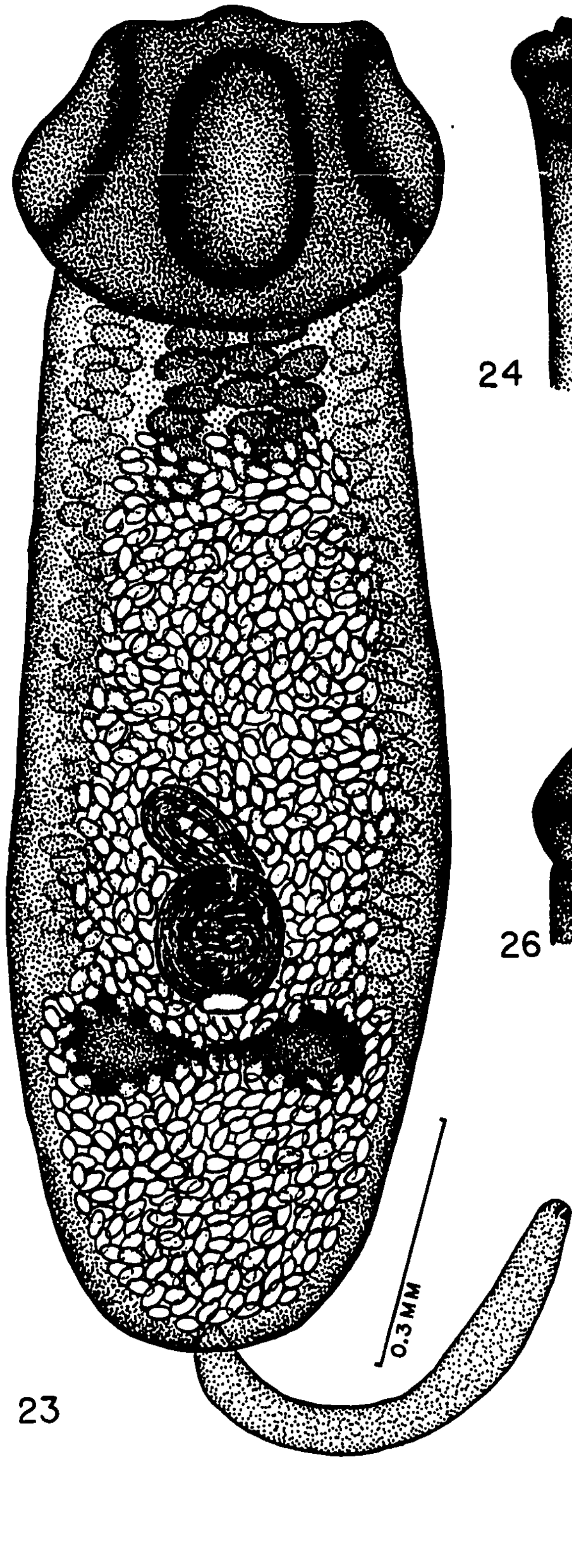
Figure 24. Scolex of freshly fixed specimen

Figure 25. Scolex of specimen fixed after remaining in distilled water for 24 hours

Figures 26 and 27. _Archigetes iowensis_, adult. (Scale as in Figure 27)

Figure 26. Scolex of freshly fixed specimen

Figure 27. Scolex of specimen fixed after remaining in distilled water for 24 hours



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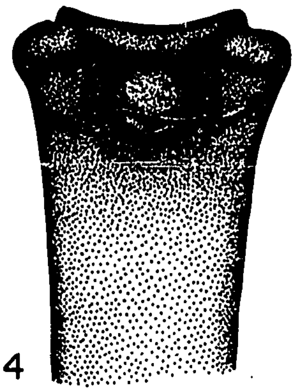

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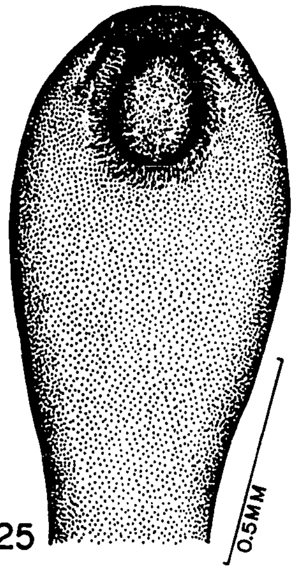

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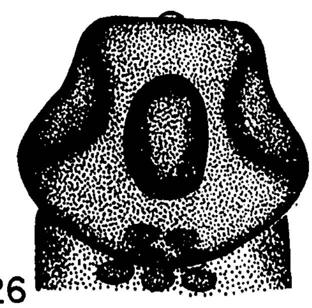

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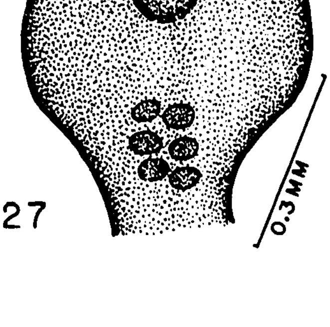

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Plate VI

Figure 28. *Monobothrium* sp. in situ, carp intestine

Figure 29. *Archigetes iowensis* in situ, carp intestine

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